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SERUM THERAPY

BY THE SAME AUTHOR.

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SERUM THERAPY

BACTERIAL THERAPEUTICS

AND

VACCINES

BY

R. T. HEWLETT, M.D., M.R.C.P., D.P.H. (LOND.)

PROFESSOR OF GENERAL PATHOLOGY AND BACTERIOLOGY, KING'S COLLEGE, LONDON
PHYSICIAN TO THE SEAMEN'S HOSPITAL, GREENWICH
LECTURER ON BACTERIOLOGY, LONDON SCHOOL OF TROPICAL MEDICINE
FORMERLY BACTERIOLOGIST TO THE JENNER INSTITUTE
OF PREVENTIVE MEDICINE, LONDON

LONDON
J. & A. CHURCHILL
7 GREAT MARLBOROUGH STREET
1903

75m0

PRINTED BY
SPOTTISWOODE AND CO. LTD., NEW-STREET SQUARE
LONDON

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PREFACE

In this little book I have endeavoured to give a concise account of the preparation of, and the treatment of disease with, anti-toxins and anti-sera, and various other substances, vaccines, &c., obtained from bacterial cultures and the like. In all cases brief directions are given for the making and testing of the various preparations; these are not in any way complete, since many small details, so conducive to success, can be learnt only by practical experience in the laboratory.

In order to render the subject matter more complete, short descriptions of certain substances of a somewhat allied nature, such as the typhoid extract of Jez, cancroin, &c., together with blood-transfusion and saline infusion, have been included.

Further information upon immunity, and full details for the isolation and cultivation of the various micro-organisms mentioned, will be found in the writer's 'Manual of Bacteriology' (J. & A. Churchill, 2nd ed., 1902).

I have to thank Messrs. ALLEN & HANBURYS for the loan of block illustrating anti-toxin syringes.

KING'S COLLEGE, LONDON,
July 1903.

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SERUM THERAPY

CHAPTER I.

INTRODUCTION — IMMUNITY — EHRLICH'S 'SIDE-CHAIN' THEORY—ANTI-TOXIN FORMATION—ANTI-MICROBIC SERA—HÆMOLYSIS

INTRODUCTION

The fascinating study of the production of immunity or insusceptibility to morbid conditions is one that dates back to remote times, though it is true that in the early and middle ages the insusceptibility aimed at was mainly against poison. It can hardly be doubted also that the ceremony of blood brotherhood, the history of which is lost in the mist of the past, was one in which by the interchange of the blood of two individuals something of their natures was supposed to be transferred one to the other so that they would in the future act together in harmony and to their common good. During the middle ages injections of blood were given for various purposes; among others, lamb's blood was used in the treatment of leprosy. It was not however until comparatively recent times that the study of the nature and production of immunity or insusceptibility to disease was systematically and scientifically pursued.

The first landmark which stands out pre-eminent above others was the discovery, by Jenner, a century ago, of the protective action of vaccinia against small-pox. The establishment of the truth of the germ theory of disease was almost a necessary preliminary to further advancement, and we have the remarkable work of Schwann, Tyndall, Davaine, Pasteur, Lister, and Koch in this direction. To Pasteur was reserved the honour of first artificially producing immunity to infective disease—namely, in anthrax, chicken cholera, and, best known of all, in rabies. In the Pasteurian method the *materies morbi* is artificially modified and weakened, and, on injection, causes a transient illness, which is soon recovered from, but which, for a limited period at least, protects against the disease. During the last few years, owing to the failure to obtain curative substances, similar to diphtheria anti-toxin, for cholera, plague, and typhoid fever, there has been a return to the Pasteurian method, as witness the cholera and plague vaccines of Haffkine and the typhoid vaccine of Wright. It must be clearly understood that the Pasteurian method does not cure the disease when this has declared itself; it is only a preventive of an attack. A new departure was made when Salmon and Smith in America found that the chemical products of the hog-cholera bacillus, freed from the micro-organisms themselves by filtration through porous porcelain, would produce immunity and protect an animal against injections of the living microbe. This fact, as is well known, was extended to diphtheria and tetanus through the researches of Roux, Vaillard, Behring, and others; but it was reserved for Behring to show that the immunity produced by the injection of bacterial toxins could be transmitted to a second animal by injections of the blood-serum of the first or treated one, and that this blood-serum, termed anti-toxin, could also be used as a curative agent. Although the practical application of anti-toxin treatment is so largely due to Behring and Roux, no man has done more for the study and theory of immunity than Ehrlich, whose marvellous inductions, confirmed as they have been by most beautiful and masterly experimental methods, must place him in the forefront of investigators in this difficult subject.

Immunity

In the first place, it is necessary to consider what is meant by immunity. Immunity is, briefly, insusceptibility to disease, generally to an infective disease. An infective disease is one which is caused by a living *materies morbi* or micro-organism, and is capable of being transmitted from one individual to another: it is an infection in contradistinction to an intoxication, in which the agent that causes the disease is a chemical substance, which is the product of the activity of a living organism or cell. Infective diseases include both the so-called infectious and contagious disorders, between which there is no real distinction. It is true that in many disorders regarded as infective, no causative organism is known with certainty—for example, in small-pox, typhus fever, and chicken-pox; but there can be no doubt that such are due to living organisms. Ergotism is an intoxication, and beri-beri is probably a disease of a similar nature.

It is also a striking and undoubted fact that some individuals are much more prone to the attacks of infective disease than are others.

One individual will pass through life almost untouched, a second and less fortunate one seems to contract every possible disease. Immunity or insusceptibility to infective disease is not confined to man, but is a peculiar property of all living things, both animal and vegetable. Thus the white rat and Algerian sheep are not liable to anthrax infection ; glanders, which is common in the horse, is rare among cattle, and is unknown among swine. The monkey and ox suffer grievously from tuberculous disease, which in the dog and goat is hardly ever met with. Dealing with the vegetable kingdom, it is the aim of the horticulturist to produce varieties, especially of vegetables, insusceptible to the ravages of the various diseases, bacterial and fungoid, to which they are liable. Thus there are some varieties of the potato obtained by artificial selection which are far less liable to be attacked by the dreaded potato disease, due to a fungus, than are others. Immunity or insusceptibility to disease is probably never absolute ; thus the fowl, which is almost insusceptible to the tetanus toxin, and can be given a dose which would kill hundreds of guinea-pigs or rabbits, may be tetanised by massive doses of the toxin, and an individual who has passed unscathed through epidemics of infective disease may ultimately become infected. Many varieties of this immunity or insusceptibility may be distinguished. In the first place, there is a natural and there is an acquired immunity; the former, natural immunity, is that which is preexistent or inherent in the individual species; acquired immunity, on the other hand, is that which is induced in some way. As regards natural immunity, this may be racial, appertaining to the race, for example: the black man is comparatively insusceptible to malaria and yellow fever, the result probably of the action of natural selection, and the Algerian sheep and white rat are immune to anthrax, while the European sheep and the brown rat are highly susceptible. Cases of individual immunity also occur; a small proportion of persons of the white race, for instance, seem to be insusceptible to malaria. Natural immunity is doubtless due to a number of different factors, such, for example, as the body temperature, the degree of alkalinity of the blood and tissues, the presence of substances, proteid in nature, which exert an inhibitory power on the development of, or a germicidal action upon, the specific micro-organisms, phagocytosis, and the inhibitory action of other micro-organisms. As regards the latter, Metschnikoff ascribes the immunity of animals to intestinal cholera as being largely due to the microbial flora of the intestine antagonising the action of the cholera microbes. On Ehrlich's 'side-chain' theory, to be discussed immediately, it may well be that the atomic groups which unite with the toxin molecule and so give rise to the toxic action are absent, and the cells are, therefore, immune.

It is, however, acquired immunity, and especially its artificial production, that is of chief interest. Acquired immunity is frequently produced as a result of an attack of disease; it may be extremely marked and lasting, as in the case of small-pox, yellow fever, and scarlet fever, or is transient and ill-defined, as in diphtheria, erysipelas, and pneumonia. That a transient immunity is produced in the last-named diseases seems certain, for it is difficult to conceive that the disease processes would otherwise come to an end.

There are also many artificial methods of producing an acquired immunity, the chief of which are the following.

1. By treatment with a modified and attenuated or less virulent form of the infective agent. This is the Pasteur system of vaccination, and is applied in anthrax and other diseases. The anthrax vaccine is prepared by

growing the anthrax bacillus at a high temperature or in the presence of small quantities of antiseptics, that is to say, under unfavourable circumstances, whereby its virulence is diminished to such an extent that a transient illness only and not death is produced on inoculation. The Pasteur system of inoculation for hydrophobia is probably based on the same principle, and it can hardly be doubted now that vaccinia is modified variola.

2. Secondly, immunity may be induced by cautious treatment with killed cultures, or with bacterial toxins. The typhoid and plague vaccines are killed cultures of the respective microbes, while by treating an animal with gradually increasing doses of tetanus or diphtheria toxin, immunity to tetanus or diphtheria may be induced,

3. Thirdly, by injections of the blood-serum of an animal which has been treated by the last method, that is, with sterilised cultures or with bacterial toxins.

4. Fourthly, in rare instances by treatment with sterilised cultures or toxins of a different species ; thus sterilised cultures of the *bacillus pyocyaneus* will protect against anthrax, and of the *bacillus prodigiosus* against the *bacillus coli communis*. Emmerich and Loew have iso-

lated from cultures of the *bacillus pyocyaneus* an enzyme-like body which possesses protective and curative properties against diphtheria.

Ehrlich by his classical experiments with abrin and ricin, two toxic proteids obtained from the jequirity and castor oil seeds beans respectively, showed that acquired immunity is of two kinds, one 'active,' as he termed it, of long duration and resulting from an attack of the disease or vaccination with a modified virus and not transmissible to the fetus; the other, 'passive immunity,' resulting from the inoculation of an animal with the blood-serum derived from another animal immunised by the injection of bacterial toxins. 'Passive immunity' is soon lost, but while present is transmitted to the fetus.

Anti-Sera

If an animal be treated for a long period of time with bacterial toxins or with bacterial cultures, the animal acquires a high degree of insusceptibility and its blood-serum may be used to confer immunity upon, or to cure disease in, a second animal. The blood-serum of an animal so treated and possessing these properties is termed generally an anti-toxin or an anti-serum. There are two classes of curative sera, the one antagonising the bacterial toxins such as diphtheria and tetanus anti-toxins, to which the term anti-toxin is alone strictly applicable, the other antagonising the microbes, killing or otherwise disposing of them. This latter class may be termed anti-microbic sera; such are anti-streptococcic and anti-plague sera. In all cases perhaps both anti-toxic and anti-microbic substances are present, but in an anti-toxic serum the anti-toxic constituent is relatively in very large excess, while in an anti-microbic serum it is almost negligible. The process of immunisation of the animal for the production of anti-toxin is an extremely tedious one, extending over months. At first minute doses of the toxin or culture are administered, and as the animal becomes accustomed to the treatment, the dose which is administered, either subcutaneously or intravenously, is gradually and progressively increased. From time to time tests are made as to the protective power of the serum, and when this has reached a sufficiently high degree the animal is bled, the blood allowed to clot, and the serum bottled for use.

Nature and Formation of Anti-Bodies

What is the nature of these anti-bodies in the anti-sera? how are they formed? and how do they act?

In order to answer fully these questions it will be necessary in the first place to discuss the mode of interaction which takes place between toxin and anti-toxin. Toxin and anti-toxin antagonise each other, and at one time, especially under the influence of certain experiments by Büchner, it was believed that this interaction was a vital one, the anti-toxin in some way rendering the cells insusceptible to the toxin. The quantitative experiments of Ehrlich with diphtheria toxin, and the filtration ones of Martin and Cherry with the same, and also with snake venom, seem to prove that the interaction between toxin and anti-toxin is a chemical combination analogous to the combination of an acid with a base—for example, oxalic acid and potassium carbonate—an innocuous compound being formed, and that the interaction follows the ordinary rules of chemical combination. Thus cold retards while concentration and warming hasten the combination, and a lapse of time is required for the complete interaction to take place. A mixture of toxin and anti-toxin kept in contact for a short time may still be toxic, but after a longer time becomes non-toxic. If a certain definite amount of diphtheria anti-toxin, which may be termed one immunising unit, be mixed with varying quantities of a given toxin, an amount of the toxin which is exactly neutralised by this amount of anti-toxin, that is, by one immunising unit, can always be determined. Ehrlich found, for example, on using one-tenth of an immunising unit of anti-toxin, that the quantity of a certain toxin which was exactly neutralised was 0·24 c.c. On making an analogous determination with *ten* times the amount of anti-toxin—that is, with one immunising unit—the maximum amount of toxin which could be given with it without producing any effect, that is was exactly neutralised, was found to be 2·4 c.c., just ten times the previous amount. To put it algebraically, let AT = one unit of anti-toxin, T = toxin in cubic centimetres, and 0 = exact neutralisation, there being an excess neither of anti-toxin nor of toxin, then in the first instance

$$(1) \quad \frac{AT}{10} + 0{\cdot}24\ T = 0,$$

and in the second instance

$$(2) \quad AT + 2{\cdot}4\ T = 0.$$

The amount of a toxin which when mixed with the unit of anti-toxin is just neutralised is termed by Erhlich the L_0 dose ($L =$ *limes* $=$ boundary, *i.e.* between life and death).

It is also possible to mix anti-toxin with an excess of toxin to the extent that a simple lethal dose of toxin remains unneutralised. This simple lethal dose of toxin is just sufficient to cause the death of a guinea-pig weighing 250 grams on the fourth or fifth day after inoculation, and the amount can be experimentally determined with considerable accuracy. This has been termed by Ehrlich the L_+ dose. In the case of a certain toxin, on using one-tenth of an immunising unit of anti-toxin Ehrlich found the L_+ dose was 0·037 c.c., but using ten times the amount of anti-toxin, *i.e.* one immunising unit, the L_+ dose of toxin was only 0·26 c.c. and not 0·37 c.c. ten times the previous amount, as in the case of the L_0 dose. Obviously in the last example ten times the amount or 0·37 c.c. would leave *ten* lethal guinea-pig doses free instead of *one*. Or, to express it algebraically, let $AT =$ one unit of anti-toxin, $T =$ toxin, in cubic centimetres, and $LD =$ the simple lethal dose, then in the first instance

$$(3) \qquad \frac{AT}{10} + 0{\cdot}037\ T = 1\ LD,$$

and in the second instance

$$(4)\quad AT + 0{\cdot}26\ T = 1\ LD.$$

Evidently if ten times the amount of toxin had been used

$$(5)\quad AT + 0{\cdot}37\ T = 10\ LD.$$

An example will make this clear. If ten equivalents of NaOH be mixed with eleven equivalents of HCl, one equivalent of HCl will remain unneutralised. If, however, the same amount of acid is to remain free on using ten times the amount, or 100 equivalents, of alkali, there must be added not 10 × 11, or 110, but only 101 equivalents of acid, HCl. To express it in the form of an equation, in the first instance

$$(3')\quad 10\,NaOH + 11\,HCl = 10\,NaCl + \\ 1\,HCl \text{ left unneutralised};$$

in the second instance if ten times the amount of alkali and of acid had been used

$$(4')\quad 100\,NaOH + 110\,HCl = 100\,NaCl + \\ 10\,HCl \text{ left unneutralised}.$$

In order that only one equivalent of HCl shall be left unneutralised, the amounts to be used must evidently be

$$(5')\quad 100\,NaOH + 101\,HCl = 100\,NaCl + \\ 1\,HCl \text{ left unneutralised}.$$

This shows that the neutralisation of toxin by anti-toxin follows the rules of chemical combination.

In Martin and Cherry's experiments, mixtures of toxin and anti-toxin were filtered through a gelatin-coated Chamberland filter after varying periods of contact. The gelatin-coated filter, devised by Martin, consists of a Chamberland porcelain filter which has been soaked in melted gelatin. This renders the pores very fine, so fine indeed that albumin will not pass through, presumably because its molecule is too large to do so; substances, however, which have a smaller molecule, such as sugar and also bacterial toxins, still pass through at a pressure of 50 atmospheres. Brodie has shown that anti-toxin does not pass through such a filter and when anti-toxic serum is filtered through gelatin the whole of the proteids, and together with them all anti-toxic virtue, are absent from the filtrate. As the toxin is not held back by the filter, whereas the anti-toxin is, this provides a physical means of separating them, *provided they have not reacted upon each other.* Martin and Cherry mixed diphtheria toxin with sufficient anti-toxin to more than completely neutralise all the toxin. This mixture was allowed to remain in contact at 30° C.

for two hours, and was then filtered through the gelatin filter: the filtrate was found to be quite innocuous. If the toxin had remained unaffected, it presumably would have passed through the filter; as it did not do so the conclusion is that it had entered into some sort of chemical combination with the large anti-toxin molecules. Another method was employed with snake venom. One of the toxic constituents of snake venom may be heated to 90° C. without injury, whereas the snake-venom anti-toxin, or anti-venin, is rendered inactive by heating to 68° C. for ten minutes. Martin and Cherry made mixtures of anti-venin and venom, pipetted off small portions at stated intervals, heated them at once to 68° C. to destroy the anti-toxin, and injected into animals. It was found that when the anti-venin and venom were kept in contact for only a short time—two minutes to ten minutes, according to the amount of venom—death ensued; whereas, when kept in contact for a longer period, the animals in all cases lived, showing that, as for all chemical combinations, time is an important factor. These experiments seem to prove that the neutralisation of toxin by anti-toxin is due to a chemical union or combination.

The toxic action of toxin upon bioplasm

would also seem to be due to a chemical union between the two ; and Ehrlich assumes that the toxin molecule possesses two different combining groups : one, which may be designated the 'haptophore' group, conditions the union with the cells (and also with anti-toxin), while the other, which may be designated the 'toxophore' group, is the cause of the toxic action. The cause of toxic action is the presence of 'toxophile' groups in the cells which unite with the toxophore groups of the toxin. If toxophile groups be absent, the toxophore groups of the toxin are unable to act, and no toxic action ensues. A diagram may make this clearer. In fig. 1 the cell is shown with protuberances to represent the haptophile and toxophile groups respectively, and the toxin with depressions for its haptophore and toxophore groups. It will

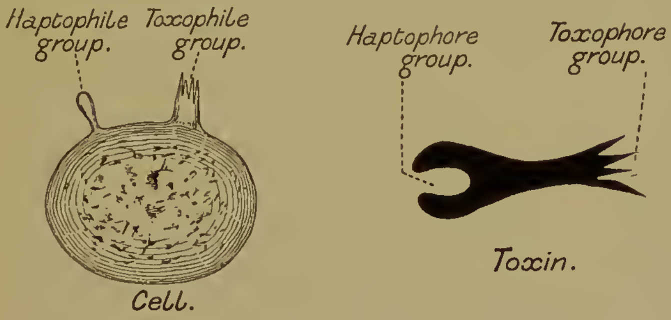


FIG. 1.—DIAGRAM TO REPRESENT THE COMBINING GROUPS OF THE CELL AND OF THE TOXIN RESPECTIVELY.

be noted that the protuberances representing the haptophile and toxophile groups of the cell are shaped so that they would exactly fit into the depressions representing the haptophore and toxophore groups respectively of the toxin—that is, the cell and the toxin could unite (see also fig. 3, p. 24). Ehrlich suggests that the haptophile and toxophile groups subserve normal functions in the animal organism, and that they only incidentally, and by pure chance, possess the capacity to unite with this or that toxin, for it is inconceivable that these atomic groups should exist simply for the purpose of fixing various toxins. Not only does toxin unite with the cells *in vivo*, but also *in vitro*. Thus tetanus toxin has a great affinity for the nervous tissues, and if an emulsion of tetanus toxin and fresh guinea-pig brain be prepared, so firmly is the toxin anchored to the nerve cells that the mixture is non-toxic, and can be injected into a guinea-pig without harm.

That toxin unites chemically with the cells seems, therefore, to be certain, whereas the alkaloids, anilin dyes, &c. form a very unstable combination, if one at all, and can be removed by the action of simple solvents such as alcohol and ether. The union of these substances with bioplasm seems to be of the nature of a 'solid solution' analogous to that of the constituent metals in an alloy.

Ehrlich's 'Side-Chain' Theory

We are now in a position to formulate Ehrlich's 'side-chain' theory. Ehrlich assumes that the living matter, protoplasm or bioplasm, of the living cells of the animal body, consists of a huge molecule or group of molecules with a number of atomic groupings, or, as they are termed by the organic chemist, side-chains which are ready to enter into combination with other suitable atomic groups should the latter happen to be present, under the requisite conditions. Thus in fig. 2, a cell is depicted with protuberances, of various shapes. These are the 'side-chains' which will only unite with other side-chains with which they have an affinity. The free bodies represent the latter, each will unite with the cell only by the protuberance, *i.e.* side-chain, to which it is apposed, as is indicated by

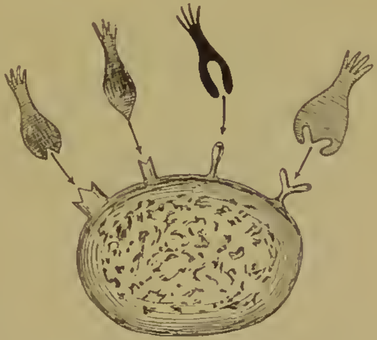
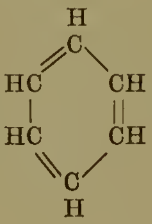


FIG. 2.—DIAGRAM TO REPRESENT THE CELL WITH ITS VARIOUS COMBINING GROUPS OR SIDE-CHAINS. (After EHRLICH.)

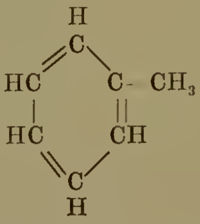
the varying shapes. These free side-chains might belong to various food-stuffs, toxins, &c.

As a simple example of side-chains the chemical nature of the benzene ring may be considered. This is composed of 6 atoms of carbon and 6 atoms of hydrogen, linked together to form a closed chain or ring, thus:[1]



= Benzene, C_6H_6

If into one of the CH groups composing this ring a methyl group is introduced by replacing the hydrogen H with methyl CH_3, the following grouping is obtained:

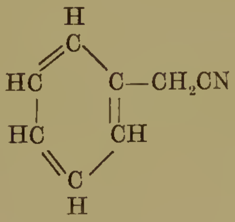


= Methyl benzene or toluene, C_7H_8

The methyl group, CH_3, in this compound forms the side-chain and will readily unite with other atoms or atomic groups. If, for example, chlorine be allowed to act upon it, the compound C_7H_7Cl is formed, and on treating this with potassium cyanide a cyanogen group may be introduced, leaving all the rest intact, thus forming benzyl cyanide:

---

[1] This is the original conception of the constitution of the benzene ring due to Kekulé, and suffices for our purpose, though it is to be noted that other constructions have been formulated. See *Encyclopædia Britannica*, 10 ed., art. 'Chemistry.'



= Benzyl cyanide, C_7H_7CN

The side-chain may be defined as an atomic group, the carbon atom of which is itself attached to one of the carbon atoms of a compound having a ring structure, either the benzene ring, which has been taken as an example, or any other ring.

Extending these ideas to living matter, Ehrlich conceives, to give his own words, ‘the protoplasm molecule as being constituted on the basis of (1) a central “functioning nucleus” of which the structure represents that adapted to discharge the specific functions characteristic of the cell, and (2) depending upon this nucleus, and providing for its sustenance certain “nutritive side-chains,” which, in accordance with the food requirements, will be quantitatively and qualitatively different; then the atomic grouping of these side-chains is so constituted that they are able to fix to themselves certain definite food-stuffs, important for the cell-life, and in consequence these food-stuffs must on their part also contain atomic groups, possessed of a maximum affinity to the side-chains. The relationship of each functioning fixing group of the corresponding groups—*i.e.* those of the food-stuffs and those of the side-chains of the cell—must be specific. They must be adapted to one another as, *e.g.*, a male and female screw (Pasteur), or a lock and key (Fischer). From this point of view we must contemplate the relation of the toxin to the cell. The relation between toxin and cell ceases to be shrouded in mystery if the view be adopted that the haptophore groups of the toxins are molecular groups fitted to unite alike both with the side-chains of the cells and with the anti-toxins, and that it is by their agency that the toxins become anchored to the cell.'[1] Ehrlich then conceives that the toxins become anchored to the cells by means of their haptophore groups, and should the cells not possess side-chains[2] which

---

[1] Croonian Lecture, Royal Society, London, 1900.

[2] Ritchie has pointed out that strictly it is incorrect to speak of the 'side-chains' of the cell; such side-chains belong to *molecules* within the cell. The term 'receptor' would be a better one.

'fit' these haptophore groups, the toxophore groups cannot become fixed to the cell, which therefore suffers no injury—that is, the organism is naturally immune. The haptophore groups would seem to act especially in bringing definite areas of the cell within the sphere of influence of the toxophore groups, and there is considerable difference in the behaviour of the haptophore and toxophore groups, as is shown by the following experiments of Dönitz and Heymans. If an animal be injected with suitable doses of diphtheria or of tetanus toxin, there ensues an incubation period of some hours, perhaps of a day or two, during which the animal remains perfectly well and unaffected by the toxin. If this dose of toxin be injected into the circulation, and *immediately afterwards* a neutralising dose of anti-toxin, no symptoms ensue, the neutralising dose of anti-toxin is able to render all the toxin innocuous. If, however, the neutralising dose of anti-toxin be injected not immediately but a few—seven or eight—minutes after the injection of the toxin, death occurs exactly as if no anti-toxin had been given. If the animal be bled immediately after the injection of the toxin, its blood being replaced by fresh blood, it still dies, so rapid is the fixation of the toxin by the tissues. The toxin held fast in the tissues is, however, still able to be withdrawn from them if a *large* dose of anti-toxin be injected, and not the simple neutralising dose. The explanation of these phenomena is that the haptophore group comes into action *immediately after* injection into the organism and so anchors, but not indissolubly, the toxin molecule to the cell, while in every toxin, with the exception of snake venom, the toxophore group does not come into activity until after the lapse of a longer or shorter incubation period.

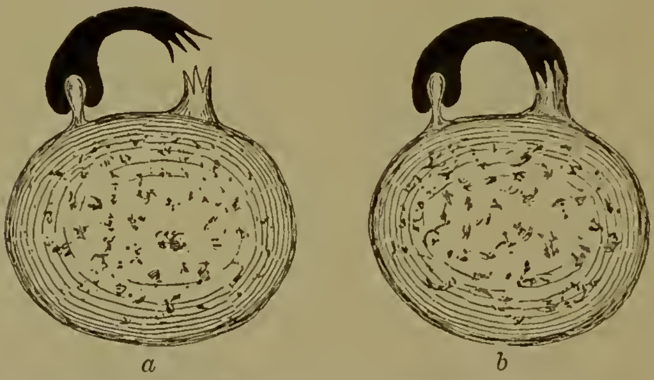


FIG. 3.—DIAGRAMMATIC SCHEME TO REPRESENT THE UNION OF TOXIN (BLACK) WITH THE CELL.

This is shown diagrammatically in fig. 3, in which the union of toxin with the cell is depicted. In *a* the toxin is represented as anchored to the cell by the union of the haptophore and haptophile groups (*cf.* fig. 1, p. 17), the toxophore group being still unattached. This is the stage in which, though the toxin has entered into union with the cells, there is as yet no toxic action, *i.e.* it is the incubation stage. In *b* the toxophore group of the toxin has now united with the toxophile group of the cell, and symptoms then ensue.

Formation of Anti-Toxin

The formation of anti-toxin can now be explained. If an animal be injected with a sub-lethal dose of toxin, the toxin becomes united by its haptophore groups to definite side-chains of the cell bioplasm. The union is a firm and enduring one, and the side-chains involved cannot exercise their normal physiological functions while this union lasts. As Ehrlich puts it, they are shut out from participating in the physiological sense in the life of the cell, and a defect has thereby been created. Now Weigert has enunciated and worked out the theory that such a defect is replaced by regeneration. Therefore new side-chains, similar to those which have been thrown out of action by the union with the toxin, are reproduced, and if more toxin be injected, again unite with it, and this union of side-chains with toxin, and regeneration of the side-chains may be repeated again and again, and the cells become educated, as it were, to reproduce the necessary side-chains in ever-increasing quantity. This accounts for the immunity which may be induced by gradually increasing doses of toxin. Whereas at first the cells possess comparatively few of the side-chains in question, and a small amount of toxin would therefore create a serious defect or lesion, when these side-chains have become very numerous, much more toxin may be injected without injury—that is, an immunity exists. But Weigert has shown that simple replacement of the defect does not take place, the compensation proceeds far beyond the necessary limit—until at last the side-chains are produced in such excess that the majority are no longer capable of remaining attached to the cells, but become free in the blood; this excess of side-chains in the blood is anti-toxin. The anti-toxin represents the side-chains reproduced in excess during regeneration, and therefore pushed off from the bioplasm of the cells, and so coming to exist in a free state in the blood.

The secretory nature of the formation of anti-toxin has additional support from the experiments of Salomonsen and Madsen, who have shown that pilocarpine, which augments the secretion of most glands, produces in immunised animals a rapid increase in the amount of anti-toxin in the blood. In certain individuals, and in horses also, a small amount of diphtheria anti-toxin seems to be *normally* present, suggesting that anti-toxin is a normal constituent of the body, which becomes increased as the result of the treatment. The mode of action of anti-toxin in neutralising toxin is also rendered clear. When the anti-toxin is injected, it combines at once with the toxin by means of the haptophore groups of the latter, and so prevents the toxin from becoming attached to the cells, and from exerting its toxic action through the toxophore groups.

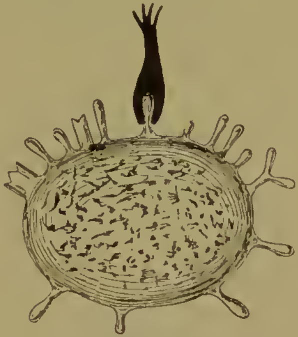


Fig. 4.—First Stage in Anti-toxin Formation. (After Ehrlich.)

These stages in anti-toxin formation are represented diagrammatically in the following figures, and serve to visualise this difficult subject. In fig. 4 the first stage is shown. Toxin has been injected, and has united with the cell bioplasm, thereby creating a defect. The cell responds to this by creating fresh side-chains of the same nature as those affected. Fig. 5 shows the condition when more toxin has been injected.

A further defect has been created, and now the cell begins to respond by an excessive formation of the side-chains affected, as is shown in fig. 6.

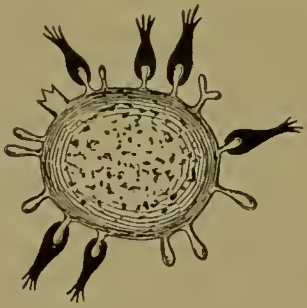


Fig. 5.—Second Stage in Anti-toxin Formation. (After Ehrlich.)

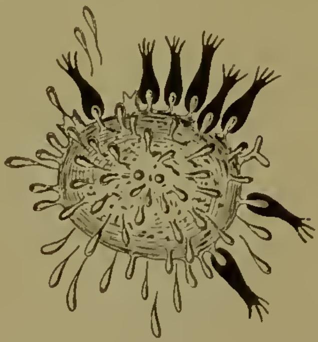


Fig. 6.—Third Stage in Anti-toxin Formation. Anti-toxin beginning to be formed. (After Ehrlich.)

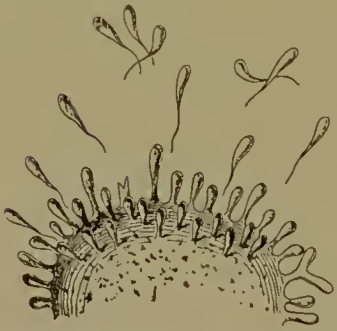


Fig. 7.—Fourth Stage in Anti-toxin Formation. Anti-toxin free in the Blood. (After Ehrlich.)

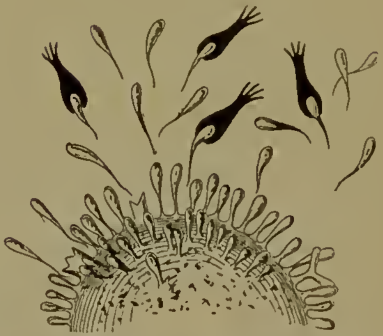


Fig. 8. — Neutralisation of Toxin by Anti-toxin in the Blood. (After Ehrlich.)

Here the side-chains are being generated in such excess that they can no longer all remain attached to the cell, and are beginning to be cast off and to be present in the blood. It is these free side-chains which Ehrlich believes to constitute anti-toxin, and fig. 7 represents the condition of the blood of an animal which is freely producing anti-toxin.

Fig. 8 represents the effect of injecting toxin into an animal in which anti-toxin is present. The toxin and free anti-toxin combine by the union of their haptophore and haptophile groups, and consequently the toxin cannot become anchored to the bioplasm, and the toxophore group is unable to exert its toxic action (*cf.* fig. 1, p. 17).

Anti-Microbic Sera

These facts hold good for diphtheria and tetanus, which seem to be different from most of the other infective diseases, typhoid, cholera, plague, streptococcic and staphylococcic infections, Malta-fever, &c. The former are essentially 'intoxication' diseases, diseases produced by the absorption of soluble chemical poisons, while the micro-organisms themselves remain for the most part localised. In typhoid fever, plague, pyæmia, &c., on the other hand, the toxins are apparently to a large extent inherent to the bioplasm of the bacterial cells. If the diphtheria or tetanus bacillus be cultivated in nutrient-bouillon and if, after the requisite time, the culture be filtered through a Chamberland filter so as to remove the micro-organisms, the filtrate will be found to be highly toxic, a single drop of the diphtheria filtrate being sufficient to kill twenty guinea-pigs, while of the tetanus filtrate fifteen drops would destroy a thousand guinea-pigs. Carry out the same experiment with typhoid, plague, &c., and the filtrate will be found to be almost innocuous even in doses of several cubic centimetres. An animal may be rendered highly immune or insusceptible to the typhoid bacillus or cholera vibrio, by carefully graduated and increasing doses of cultures of these organisms, commencing perhaps in the first instance with killed cultures and afterwards employing the living organisms, yet its blood-serum has only comparatively feeble *protective or curative properties.* It is true that a very small amount of the serum may protect against a single or two or three lethal doses of the organism, but the amount of the serum required to protect is in no way proportional to the number of lethal doses of the organism. For example, if 0·005 c.c. of cholera serum, the serum of an animal immunised by repeated injections of the cholera vibrio, will just neutralise 5 milligrams of cholera culture, injected into the peritoneal cavity of a guinea-pig, three times the amount of serum, or 0·015 c.c., will probably not protect against three times the lethal dose, or 15 milligrams, of cholera culture, and as stated above, when a few lethal doses have been reached, it is impossible to save the animal, however much serum is administered. How can this extraordinary phenomenon be explained? If about half an hour after the mixture of microbes and serum has been injected into the peritoneal cavity of the guinea-pig a microscopical preparation of the peritoneal exudate be made, it will be found that the microbes are in all stages of dissolution —that is to say, the serum causes the solution of the microbes and so destroys them. This is termed bacteriolysis, and the phenomenon is known as Pfeiffer's reaction, after its discoverer. Metschnikoff subsequently showed that the reaction would occur *in vitro* if to the mixture of microbes and serum some of the *fresh* peritoneal exudate of a *normal* guinea-pig were added. Bordet afterwards found that the reaction occurred *in vitro* in the mixture of microbes and immune serum *alone*, provided that the serum were perfectly fresh, the serum becoming inactive *in vitro* a short time after being withdrawn.

Evidently therefore two substances at least are concerned in the reaction, one a specific immunising body, different for each microbe, and found in the serum only after treatment of an animal with the particular microbe, the other present in normal serum, but in small amount, and rapidly undergoing destruction after the withdrawal of the blood from the animal. The former has been termed immune body, the latter addiment, complement, or alexin. We can now understand how it is that anti-typhoid or anti-cholera serum is only capable of neutralising at most a few lethal doses of the typhoid or cholera microbe, and possesses little curative power. In the immune serum only a *portion* of what is required is present, and the highly unstable complement is needed in addition to bring about the bacteriolysis or solution and destruction of the microbes. This last is restricted in amount, so that there is a limit to bacteriolytic action. The manner in which the two constituents act is not known with certainty. Ehrlich assumed that for the complement to exert its bacteriolytic action it is necessary for it to become attached, or to combine, as it were, with the bacterial bioplasm, but that it cannot do this, probably because the side-chains of the two do not correspond. The immune body, on the other hand, possesses side-chains which correspond, that is can combine, with side-chains of both the complement and the bacterial bioplasm, and it serves therefore as a link, bringing about the union between complement and bioplasm. This is shown diagrammatically in fig. 9. The complement (black) is there depicted united to the cell-bioplasm by means of the intermediate link or ‘immune body’ (white).

Gruber regards the immune body as in some manner preparing the way for the action of the complement, and terms it, therefore, the ‘preparer.’

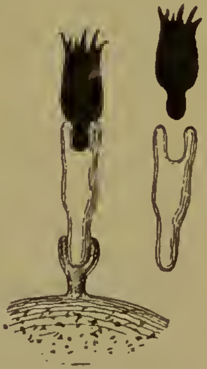


FIG. 9. — DIAGRAM TO SHOW THE UNION BETWEEN COMPLEMENT (BLACK) AND BIOPLASM OF CELL BY MEANS OF THE IMMUNE BODY (WHITE). (After EHRLICH.)

The beautiful experiments of Ehrlich and his pupils on hæmolysis or destruction of the red blood corpuscles are very suggestive as to the truth of this theory. If a guinea-pig be injected with defibrinated rabbit's blood, it will be found that the guinea-pig's blood-serum after this treatment possesses marked hæmolytic action (*i.e.* solution of the red-corpuscles) upon the red corpuscles of the rabbit, a power which it did not previously possess. If the hæmolytic serum be heated to 55° C., it is rendered inactive and does not hæmolyse, but it can be rendered active again by the addition of normal guinea-pig serum, and Ehrlich and Morgenroth explain the phenomenon of hæmolysis in this case as being due to the interaction of two substances, one specifically active and resistent, the immune body, and the other highly unstable, the addiment or complement. The phenomenon is exactly comparable to bacteriolysis.

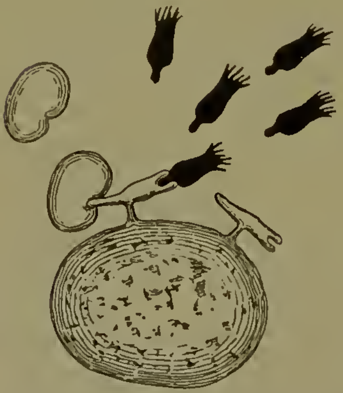


Fig. 10.—An Immune Body or Connecting-link, having two Affinities. (After Ehrlich.)

In some cases the intermediate link (immune body) has two affinities (fig. 10), in others three.

The hæmolysins, as they are termed, seem to be specific—that is, there is a different hæmolysin for every variety of erythrocyte. The potentialities of bioplasm seem to be endless, and Ehrlich says that 'the blood-serum is the carrier of substances innumerable as yet little known or conceived of.'

There are, it is true, certain difficulties to be accounted for before Ehrlich's theory can be accepted in its entirety—for example, it is difficult to understand why the injection of more toxin into a treated animal that has already developed a considerable amount of anti-toxin should be followed by the formation of more anti-toxin, though it is perhaps explicable upon certain facts known in physical chemistry (see Ritchie). Nevertheless, Ehrlich's theory has received a large amount of experimental support, and further work tends rather to confirm than to refute the truth of his wonderful conception.

LITERATURE

On immunity, &c., see Ehrlich, *Croonian Lecture, Royal Society, London*, 1900; Ritchie, *Journ. of Hygiene*, vol. ii., 1902, no. 2, p. 215 *et seq.*; Armit, *Brit. Med. Journ.* 1902, i. p. 784 *et seq.*; Grünbaum, *Brit. Med. Journ.* 1903, i. p. 653 *et seq.*; Hunt, *Trans. Path. Soc. Lond.*, xlvi., 1895, p. 266; Levaditi, *La Presse Médicale*, No. 70, Aug. 31, 1901, p. 109 *et seq.*; Myers, *Trans. Path. Soc. Lond.*, li. pt. iii. 1900, p. 195; Arrhenius & Madsen, Ref. in *Nature*, Dec. 4, 1902, p. 114; Hewlett, *Manual of Bacteriology*, 2nd ed. 1902. There is a good popular account by Carl Snyder ('Physiological Immunity') in *Harper's Monthly Magazine*, April 1903, p. 720.

CHAPTER II.

GENERAL METHODS FOR THE PREPARATION OF THE ANTI-SERA

From the preceding chapter it will be seen that the anti-sera are specific—that is, each serum acts only towards its own toxin, therefore anti-diphtheria serum is of use in diphtheria alone, and anti-tetanus serum in tetanus. From this it follows that each serum must be obtained by injecting an animal with the pathogenic organism or its toxin for which the serum is to be an antidote. Various reports have been made of the production of anti-toxin by the electrolysis of toxin, but the only practicable method of preparation is by the injection of an animal with toxins or cultures.

The Animal to be employed

As regards the choice of animal this depends upon circumstances. For experimental work in the laboratory rabbits may be used; but not more than about 20 c.c. of blood can be withdrawn from each animal at one time. A goat is a far more convenient animal, and 100 to 200 c.c. of blood may be withdrawn at each operation. For the preparation of the sera for therapeutic use larger animals of the equine species are always chosen—the ass, pony, mule, or horse. The ox might doubtless be used, but presents many disadvantages: it is prone to tubercle; less tractable; its blood does not coagulate so satisfactorily, and the yield of serum is less; while horse serum seems to be the least toxic of any—an important consideration when large doses are being administered.[1]

Sound animals must, of course, be chosen, though such faults as roaring, broken knees, &c. are of no moment. Before use the animal must be carefully tested with mallein and tuberculin to exclude the possible presence of glanders and of tubercle, and should be kept isolated under observation for a fortnight. On no account must a fresh animal be placed among the others under treatment until the absence of glanders or other infective disease has been proved, for the introduction of such a disease into the stable would be most disastrous; and for the same reason those animals under treatment must be kept isolated from the outer world in their own premises. The animals should be stalled in a well-ventilated, but warm and light, stable during the period of reaction after inoculation, and for a day after bleeding, but otherwise may be kept during the daytime in favourable weather in the paddocks. The animals are fed and tended on general principles; they are not subjected to any special treatment during the inoculations.[1]

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[1] Horse serum seems to be practically non-toxic except in quantities far above the ordinary therapeutic doses. Salter found that administered subcutaneously a dose of 6 c.c. of bullock serum, 9 c.c. of dog, 12 c.c. of calf, 18 c.c. of sheep, 25 c.c. of ass, and 33 c.c. of horse serum, was the minimal lethal dose respectively per kilogram of body weight for the rabbit. For different animals the toxicity varies; for the mouse and rat a quantity represented by half the body weight, for the guinea-pig $\frac{1}{25}$, and for the rabbit $\frac{1}{30}$.

'Homologous' Sera

In certain instances, for the preparation of anti-microbic sera, it may be found that an animal, such as the ape, more nearly related to man than the horse, will yield a more potent serum. Thus Sorbenheim found that an anthrax serum obtained by immunising sheep protected sheep even in small quantities, but that for rabbits it had no protective action. Ehrlich explains this on the assumption that the 'immune body' obtained by inoculating sheep does not meet with an appropriate 'complement' in the rabbit, and suggests that the non-success hitherto obtained with the anti-typhoid and anti-cholera sera in man may be due to this factor. Therapeutic sera obtained by the treatment of a species nearly related to that for which they are to be used might be termed 'homologous sera.'

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[1] See Robertson, *Trans. Path. Soc. Lond.*, xlvi. 1896, p. 297.

The Toxic Material for Inoculation

For those organisms which produce a powerful toxin, *e.g.* diphtheria and tetanus, *toxin broth* is employed for the most part for the inoculations, supplemented often by injections of the dead or living *cultures*, that is, the toxin broth together with the micro-organisms. For those organisms which do not produce any appreciable amount of toxin, the dead or living cultures are injected. In either case a virulent organism must be employed, and it must be grown under such conditions as have been proved to yield a broth or culture of maximum toxicity; these will be detailed when considering each organism. In order to prepare the toxin for inoculation, the culture, after growing for the requisite time, is filtered aseptically through a porous porcelain filter, such as the Pasteur-Chamberland or Berkefeld. The latter, perhaps, is the more convenient, as the filtration is more rapid. The apparatus may be fitted up in two ways, and other modifications will suggest themselves in special instances. In the first (fig. 11) the filter-candle (B) is attached by a piece of rubber *pressure* tubing to a short length of glass tubing, passing through a rubber cork, which stoppers the mouth of a thick glass filtering flask (C); the lower end of this glass tube should terminate just below the level of the lateral tubule of the flask. The candle is placed in a jar or cylinder (A) in which the culture is placed, the lateral

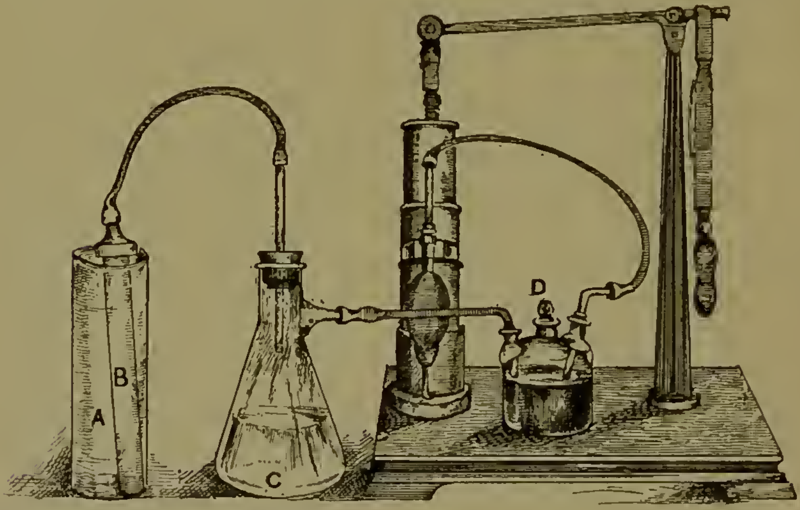

FIG. 11.—GERYK EXHAUST PUMP, ARRANGED FOR FILTRATION OF TOXIN.

tubule of the flask being connected to an exhaust pump, such as that figured, or to a water-pump. On a vacuum being created, the fluid passes through the filter-candle and collects in the filter-flask (C). In the illustration the pump is the Geryk pump, and the Wolf's bottle (D) interposed between the flask and the pump contains strong sulphuric acid, and is for the purpose of preventing the passage of water or watery vapour into the pump.

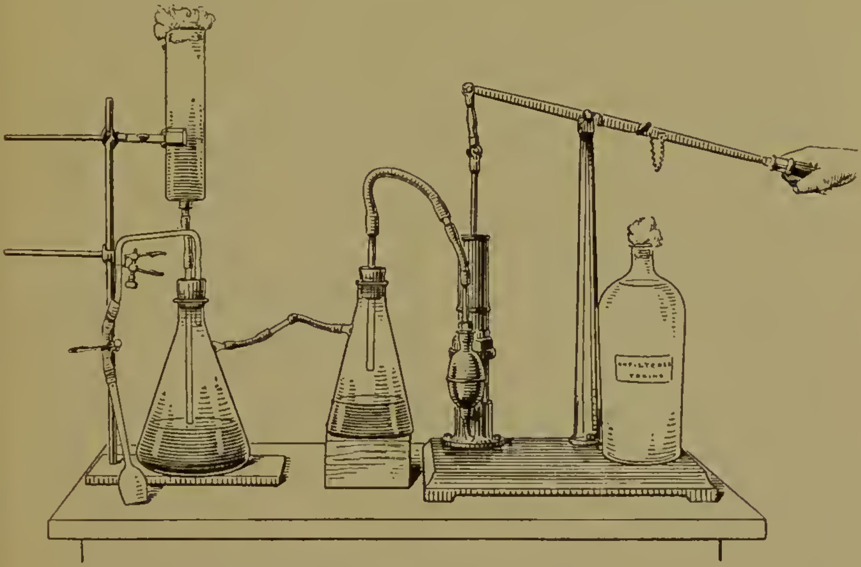

Fig. 12.—Filtration of Toxin.

In the second method of filtration (fig. 12) the Berkefeld filter-candle is contained in the reservoir above the filter-flask, being fixed by means of a nut and rubber washer; the reservoir being filled up with the culture and the filter-flask exhausted as before, the filtrate collects in the filter-flask.

If there be much suspended matter in the toxin broth, it is advisable to filter it through coarse paper previous to filtering through the porcelain filter, in order to avoid blocking the latter. The candle, rubber connections, and filter-flask before use are sterilised by steaming for some hours, and with care the filtrate may be collected aseptically, and, by clamping the rubber tube with screw clamps, may be preserved without septic change until required. Or the toxin may be preserved by the addition of 0·5 per cent. of carbolic acid or of toluol. The toxins after preparation undergo a gradual diminution in strength, and so should be used as soon as practicable after preparation. Until used they must be kept in the dark in a cool place.

When the micro-organisms are to be injected broth-cultures may be employed, or emulsions of agar, serum or other cultures are prepared with sterile physiological saline solution. In the latter instance it is as well to adopt some standard for the quantities of culture and saline solution employed, so that a rough approximation of the same amount may be gauged for dosage;

for example, the growth over the whole surface of an ordinary agar tube may be emulsified in 10 c.c. of the saline solution, or that on the surface of the agar in a plate bottle (see Plague, p. 160) in 50 c.c., the emulsion being made by means of a platinum wire needle or platinum spatula. When the dead organisms are used for inoculating, heat is generally employed to destroy their vitality. For non-sporing forms a temperature of 65° C. for 10–20 minutes usually suffices, but care must be taken that the whole mass of fluid attains and is kept at this temperature for the requisite time. In certain instances the bacterial proteins derived from the bacterial cells are made use of instead of the organisms themselves. For this purpose the organism is cultivated on a solid medium such as agar, the growth is scraped off and emulsified in distilled water. To this is then added caustic soda to the extent of 0·1–1·0 per cent., and the mixture is then boiled and filtered. To the filtrate dilute (1 per cent.) acetic or hydrochloric acid is added; this causes a precipitate of the protein (probably a body of the nature of a nucleo-proteid), which is collected on a filter-paper, washed first with slightly acid water, and then with sterile distilled water, and dried; or it may be purified by re-solution in alkali and precipitation with acid. For injection purposes it may be dissolved, with the aid of heat if necessary, in a sterile 1 per cent. solution of sodium carbonate.

During the preliminary stages of injection, until some degree of immunity has been attained, it is often advisable to diminish the toxicity of the toxin when this is a very potent one. This can be done by heating the toxin for a short time to 60°–70° C., or by mixing it with one or two times its volume of iodine solution (*e.g.* iodine 1 part, iodide of potassium 2 parts, water 300 parts).

Or some anti-toxin may be given at the same time as the injection of toxin, sufficient to partially neutralise the latter.

It seems to be a general rule that during the injections the anti-toxic or anti-microbic properties of the blood gradually increase until they attain a maximum some months (4–6) after the commencement of the treatment; they then remain at about this level for a further period of six to twelve months, but afterwards, in spite of continued injections of large doses of active toxin or culture, they gradually wane and finally almost disappear, although the animal still retains its immunity. After a period of rest, extending over a year or two, the same animal may again be employed for the production of the anti-toxin. On Ehrlich's side-chain theory Weigert has explained this fall in anti-toxic value as being due to damage done to the reparative portions of the cells which reproduce the anti-toxic side-chains. As is so common in hyperplasia and hypertrophy generally, to which the over-production of the anti-toxic side-chains has some analogy, the process after a time is apt to fail, and degeneration to ensue. This may be the case with the anti-toxic side-chains; by continual stimulation the cells become exhausted as it were, and fail to produce them; while, since they are no longer present to combine with the toxin, immunity still persists. By resting the cells, provided they have not been too much damaged, it may well be that they recover their functions, and subsequently behave as untreated cells.

'Polyvalent' Sera

Different strains or races of the same species of microbe undoubtedly vary somewhat in their pathogenic action. Thus the local reaction at the seat of inoculation may be much more marked with one diphtheria bacillus than with another, and it has been found that an anti-pneumonic serum prepared with one strain may not immunise against another strain (p. 152), and the same probably holds good for the streptococcus (p. 145). In view of this the writer has for years advocated the use of several strains of the microbe for the preparation of an antiserum, believing that such a serum would be found to be more generally efficacious than one prepared with a single strain. A serum prepared with several strains or races of the microbe is termed a ‘polyvalent’ serum.[1]

Injection of the Toxin

Toxin, in the early stages of immunisation, is usually administered by subcutaneous injection. An ordinary syringe, with glass barrel and asbestos packing for the piston, is employed, of suitable capacity—5, 10, 20, 50, or 100 c.c.—according to the dose, and graduation marks on the barrel are an advantage. The syringe is rendered aseptic by boiling in water and is completely filled with the toxin solution, which is injected into the loose subcutaneous tissue at the base of the neck just in front of the shoulder. The skin at the seat of injection should be previously prepared by shaving an area four to six inches square, washing with soap and water, and then with lysol. The needle of the syringe should be kept bright and sharp, and it may be attached to the syringe by a short length of rubber tubing, if the horse is a restive one. The injections are preferably made alternately on the right and on the left side, the skin being pinched up and the needle pushed through the skin with a quick plunge.

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[1] Grünbaum, *Practitioner*, Nov. 1902, p. 620.

A second injection should not be administered until the reaction caused by the previous one has subsided, but in the later stages, when the animal ceases to react, the injections may be given once or twice a week. The animal does not usually need further restraint than that secured by a halter, but a twitch may be necessary; more elaborate restraint in a framework with straps, &c. is only exceptionally required. In all operations the eyes of the animal should be covered with a cloth.

The temperature of the animal should be taken previous to the injection, and at intervals of a few hours after the injection, until the temperature attains and remains at the normal (for the horse = 100° F.). The effect of the injections, in the earlier stages at any rate, is to cause some fever and general malaise, during which time the animal should be kept in the stable. The injection must not be repeated until the animal recovers from its indisposition, until the temperature has remained at the normal for a clear day, and until any acute local irritation at the seat of inoculation has subsided. The second injection may equal in amount the first, and the dose should not be increased until the reaction produced becomes slight. The injections should be given in different places in order to avoid setting up too much local irritation, with perhaps cellulitis or suppuration. When large doses have been reached by subcutaneous inoculation, intravenous injection may be substituted. The needle of the syringe is passed into the jugular vein in the same manner as that described for bleeding (see below), but pointing posteriorly, *i.e.* in the direction of the blood-stream. Care must be taken to avoid injecting any air, and the toxin should be injected slowly. For intravenous injection, the toxin should always be warmed to about 37°C.

Bleeding the Animal

The serum of the treated animal having acquired the requisite potency, the animal must be bled. The best time for withdrawing blood, in relation to the injections of toxin or cultures, varies under different circumstances, and will be referred to when discussing the preparation of the individual anti-sera. Bleeding is a comparatively simple matter in the horse, in which the jugular vein runs superficially in the groove on either side towards the lower aspect of the neck. If pressure be made at the base of the neck over the vein, this will fill up and stand out and be quite evident. The skin is shaved over the course of the vein at about its middle, and disinfected by well scrubbing with soap and water and then with lysol (2 to 3 per cent.). The apparatus required are a large pointed cannula, about 6 inches in length and of $\frac{1}{16}$ to $\frac{1}{8}$ in. bore, several large filtering flasks of two to three litres capacity, glass tubing, rubber corks, &c. The cannula is carefully sterilised by boiling, and may be kept for use in a sterile glass-tube plugged with cotton-wool. The filtering flasks are fitted up as follows: the neck is plugged with a rubber cork pierced with one hole, through which a short length of glass tubing passes. Attached to the free end of the glass tube is a short length of rubber tubing, of such a size that it will fit the cannula, which is clamped at its proximal end with a screw clamp, and plugged with cotton-wool at its distal free extremity. The side tubule of the filtering flask is also plugged with cotton-wool, and the whole is carefully sterilised for several hours in the steam steriliser or autoclave. For an ordinary bleeding about six of these flasks will be required. The horse does not usually require more restraint than that employed for injection of the toxin.

The position of the vein having been ascertained by applying pressure at the base of the neck, and observing the filling up of the vein, a small longitudinal incision is made through the skin with a sharp scalpel in the prepared area. The vein being rendered prominent by pressure on its course below the seat of operation, the sterile cannula is pushed into the vein in the direction of its long axis and anteriorly—*i.e.* towards the head. When the cannula is properly in the vein the blood will run freely from it, and it is then connected to the sterile flask by the rubber tube, the wool plug being removed and the clamp unscrewed. When the flask is about two-thirds filled it is replaced by another, the rubber tube being clamped and plugged with sterile wool. Six to twelve litres of blood, according to the size of the animal, may usually be withdrawn without inducing any symptoms, and all the operations must be carried out as aseptically as possible. *The animal must not be bled too soon after the last inoculation of toxin or culture, or the serum might possess toxic qualities.*

Preservation of the Anti-Serum

After the blood has been collected, the flasks are allowed to stand in a cool place for two or three hours until clotting has occurred. It is a good plan then to give each flask a few sharp twists so as to detach the clot from the sides of the flask. The flasks are then allowed to stand in a dark, cool place for twenty-four to forty-eight hours until the serum has thoroughly separated, and the serum is then decanted off through the side tubule into sterilised bottles, in which it is kept until required. By some a small amount of an antiseptic is added—*e.g.* 0·2 per cent. carbolic acid or, preferably, 0·3 per cent. trikresol. Camphor, previously flamed to sterilise it, has also been used, but is only a feeble antiseptic.

As a slight precipitate is apt to form in the anti-serum after it has been kept for a short time, rendering it turbid, it is usual to filter the serum after it has stood for a few days, and before bottling, through a Berkefeld filter, arranged as for the filtration of toxin (p. 40).

For distribution the anti-serum must be bottled aseptically. The bottles should be of about 10 c.c. capacity and be made of a nonactinic glass—*e.g.* orange or green, and the corks are preferably of india-rubber. Both must be carefully sterilised by steaming and be kept in covered metal or glass jars until required. Generally speaking, a single therapeutic dose only is placed in one bottle, so that there can be no risk of contamination by the repeated opening of a bottle.

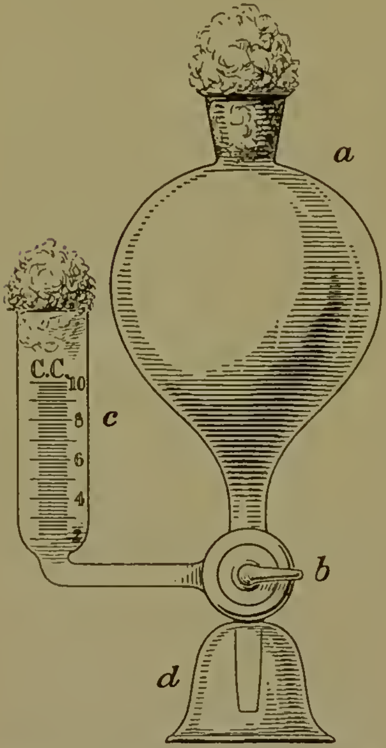

Fig. 13.—Simple Apparatus for bottling Anti-toxin.

For bottling various devices may be adopted. In the one figured (fig. 13), the whole apparatus is carefully sterilised by steaming, and the large bulb *a* filled with the serum from the stock-bottles. *b* is a two-way cock, so that the graduated side tube *c* may be filled with the serum by turning the cock one way, and by turning it the other, any requisite measured amount may be run out into the bottles, the hood *d* tending to prevent aërial contamination. Each bottle as filled with the required amount is handed to an assistant who corks it. After corking, the tops may be dipped into melted paraffin-wax to seal the bottles hermetically. Each batch as finished is at once labelled with the appropriate labels, so that no mistake can occur, and the bottles are then stored in a cool chamber in the dark. Some makers, instead of corking, use phials with long slender necks which after filling are sealed in the flame. Machines have also been devised for the process of bottling. The whole of the manipulations must be carried out as expeditiously as possible, and with the most scrupulous aseptic precautions. The bottling chamber should be used for nothing else, the walls should be tiled and the tables and floors of cement, so that the whole may be sprayed with an antiseptic. Double doors should be provided, and the ventilating inlets and outlets screened with cotton-wool, so that filtered air only is admitted. The operators should wear sterilised blouses or mackintosh ones, so that these may be swabbed down with an antiseptic, and their hands must of course be disinfected.

When the anti-serum has to be kept for any length of time, to be exported, and especially for use in hot climates, it is preferable to evaporate to dryness and to send out the solid dried serum. The evaporation is carried out *in vacuo* over sulphuric acid, and a simple apparatus for this purpose was devised by the writer (fig. 14). A large stout bell-jar with ground rim and two tubules, one on the top into which a mercurial gauge is inserted through a rubber cork, the other at the side near the base, which is plugged with a rubber cork, through which a glass tube with stopcock passes, ending flush with the inside and connected the other end by means of thick rubber *pressure* tubing to an exhaust-pump. The bell-jar stands on a stout piece of ground glass ($\frac{5}{8}$ in. thick), and a metal framework

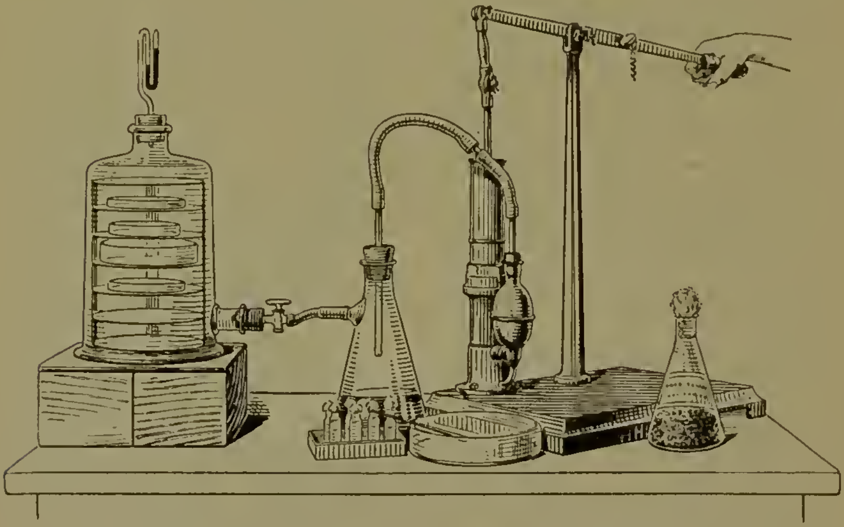

Fig. 14.—Apparatus for Desiccating Anti-toxin.

with shelves is placed inside. The shelves support shallow ($\frac{3}{4}$–1 in.) glass dishes, and there is just sufficient distance between them to admit the glass dishes.

The dishes containing strong commercial sulphuric acid and serum alternately are stacked upon the shelves, the bell-jar is placed over the whole and is then exhausted. The gauge will show the amount of the vacuum, and the exhaustion should be stopped and the stopcock closed as soon as the serum commences to bubble. All joints of the apparatus must be made tight with a luting composed either of the resin ointment of the Pharmacopœia or of a stiff paste of beeswax and Geryk pump oil mixed by gentle warming. Not only should the ground edge of the bell-jar be smeared with the luting, but when pressed home the angle between the rim and the ground-glass plate should be plastered with it. The dishes for the serum should be covered with filter-paper and carefully sterilised, and preferably should contain only a shallow layer, about $\frac{1}{4}$ in. deep. The dishes with the sulphuric acid should be half filled, and for rapid evaporation should be more numerous than the serum dishes. The apparatus used by the writer held seven dishes, each 1 inch deep over all, and 5 inches in circumference; four of the dishes contained sulphuric acid, the other three the serum. If the vacuum is not well maintained, all the joints should be gone over and luted afresh. With a shallow layer of serum, desiccation will be complete in 24–48 hours; but if the dishes be nearly filled, it will take longer, and the sulphuric acid may have to be renewed once. Warmth assists the evaporation, and the apparatus may be kept in a warm room or be placed in a warm (37°–40° C.) incubator. Special pieces of apparatus have been devised to carry out the desiccation at 40° C.

The dried serum is chipped off the dish with a sterile spatula, and is put up in sterile glass tubes. If the layer of fluid serum is shallow, the dry substance will be in thin scales very suitable for solution; but if a deep layer be evaporated, it will need pulverising in a sterile mortar before bottling. One gram or 15½ grains of the dry substance corresponds to about 10 cubic centimetres or 2¾ drachms of the fluid serum.

CHAPTER III.

GENERAL PRINCIPLES OF TREATMENT WITH ANTI-SERA—ADMINISTRATION OF ANTI-SERA—COMPLICATIONS AND SEQUELÆ OF THE TREATMENT

General Principles of Treatment with Anti-sera

It should be clearly understood and recognised that the phrase 'cure of disease' is, in an active sense, a misnomer. The actual 'healing' of a damaged tissue or restoration of function of a disordered organ is brought about by the reparative powers of the vital activities of the living matter, bioplasm, or protoplasm of the body, the *vis medicatrix naturæ.* Strictly speaking, the physician or surgeon, by his art and science, does not 'cure' in the sense of repairing actual damage; all that he does is, as far as possible, to bring about a condition of things most favourable for the exercise of its healing functions by the living matter. First of all, as far as possible, he removes the cause of the disordered condition, and then, for example, he compels rest, and applies cold or heat to an inflamed part; he administers drugs which are known by experience or experiment to modify the activities of the living matter in a particular direction, and so on. It therefore follows that if tissue damage has occurred through the action of mechanical injuries, toxic substances, and such like, the administration of drugs and similar substances will not directly repair the damage, but will only assist the curative action of the tissues. *Tissue damage is repaired by cellular action alone.*

The anti-toxins and anti-sera are able to neutralise the toxins or activities of the bacteria which produce disease, and if they be administered early enough, they will prevent the tissue damage to which the morbid symptoms are due. Anti-toxin has no more power than any other drug to repair tissue damage if this has already occurred.

Early treatment is, therefore, of paramount importance.

As will be mentioned later, the mortality from diphtheria is practically nil when the disease is treated with anti-toxin from the first day, and steadily rises on each succeeding day that the treatment is delayed.

Secondly, as has been shown in Chapter I., the neutralisation of toxin by anti-toxin is strictly quantitative—that is, a given quantity of toxin requires an equivalent amount of anti-toxin to neutralise it. Moreover, as toxin seems to combine with the tissues, and since the longer the toxin acts the more stable this combination appears to become, a relatively larger amount of anti-toxin should be administered if the disease has lasted for some time than if it has only just shown itself.

The anti-serum must be regarded as a solution of the antidotal substance in blood-serum, and in different sera the same volume of serum may contain very different amounts of the antidotal substance. The anti-serum should therefore be standardised, and the prescribed dose given.

A sufficient amount of anti-serum must always be administered. This does not depend upon its volume, but upon the amount of antidotal substance it contains.[1]

There is no risk whatever of toxic symptoms from the serum being a 'foreign' one. Salter· considers that if human beings were as susceptible as the most susceptible animal, quantities up to 200–250 c.c. of horse serum for a dose might be given to an infant without overstepping the limits of safety.

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[1] See Hewlett, *Treatment*, i. 1897, p. 173; Landau, *Die Serumherapie*, 1900.

As is stated below, the anti-sera are usually administered by subcutaneous injection; they are not absorbed by the mouth or rectum. But absorption from the subcutaneous tissues is comparatively slow, and if a rapid action be desired, as in bites of venomous snakes, or when the case comes under treatment at a late stage of the disease, recourse may be had to intravenous injection. In certain instances the anti-serum is injected into the region where the fixation of the toxin takes place, as into the central nervous system in tetanus.

Use of Normal Serum with Anti-microbic Sera

There does not seem to be any risk of injecting too much of an anti-toxic serum, but as regards an anti-microbic serum it may be useless to inject more than a certain amount. However, data are at present wanting to guide us in this matter. Wassermann has suggested that the efficacy of an anti-microbic serum would be much enhanced by the simultaneous injection of a *perfectly fresh* normal serum. The anti-microbic sera act by the union (?) of two substances, the 'immune body' of the anti-microbic serum and the 'complement' present in the blood of the individual and in freshly drawn blood. The amount of complement being limited (see p. 32), the injection of *fresh* serum, together with the anti-microbic serum, should supply additional 'complement' to increase the action of the anti-microbic serum. This method has not yet been tried practically, and there is, of course, the difficulty of obtaining *perfectly fresh* serum.

Administration of Anti-sera

The anti-toxins and anti-sera are usually administered by subcutaneous injection. Various statements have been made and reports are frequently published in the medical journals of the successful administration of anti-toxin by the mouth or rectum. In some experiments made by the writer[1] it was found, however, that, using guinea-pigs and rabbits, both diphtheria and tetanus anti-toxins were completely unabsorbed when given by the mouth or rectum, and therefore this mode of administration must be condemned as being perfectly useless.

The syringe employed for the injection usually contains ten cubic centimetres, the barrel being made of glass and the piston of asbestos, so that the whole may be sterilised by boiling (figs. 15 and 16). Other forms of syringe are also to be had; one is made entirely of glass, barrel and piston, and from the aseptic point of view is a most desirable form, but is of course more liable to breakage. The Koch form of syringe (fig. 17, p. 64) may also be employed, but is not nearly as convenient.

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[1] *Trans. Path. Soc. Lond.* liii. 1900, pt. ii. p. 220.

In a new syringe with asbestos piston, or one which has not been used for some time, the piston may work loose owing to contraction of the asbestos, but the latter will soon swell when placed in water or during sterilisation. There is usually a small nut at the end of the piston rod, just below the handle, which can be screwed up, and by compressing the asbestos plug enlarges its diameter, and by this means the tightness of the piston may be adjusted and the proper fit obtained.

For sterilising the syringe and needle, boiling should always be employed, though, if desired, a previous soaking in an antiseptic may be given; but this should always be followed by boiling, or at least by rinsing in sterile water, as the presence of any antiseptic is undesirable owing to the tendency of most antiseptic substances to precipitate blood-serum. For boiling, an

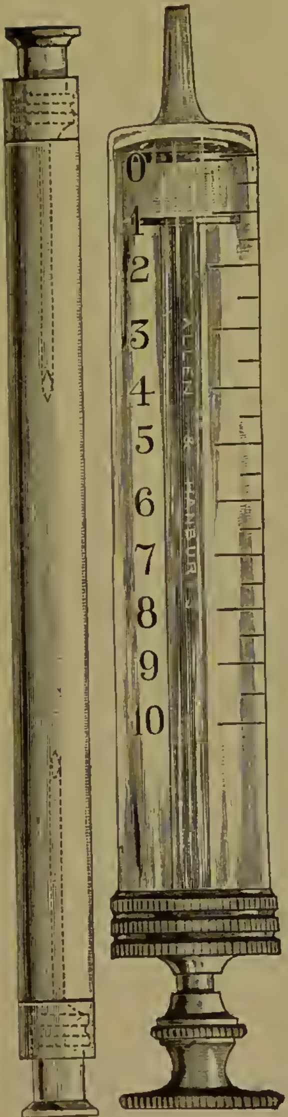

Fig. 15.—Anti-toxin Syringe.

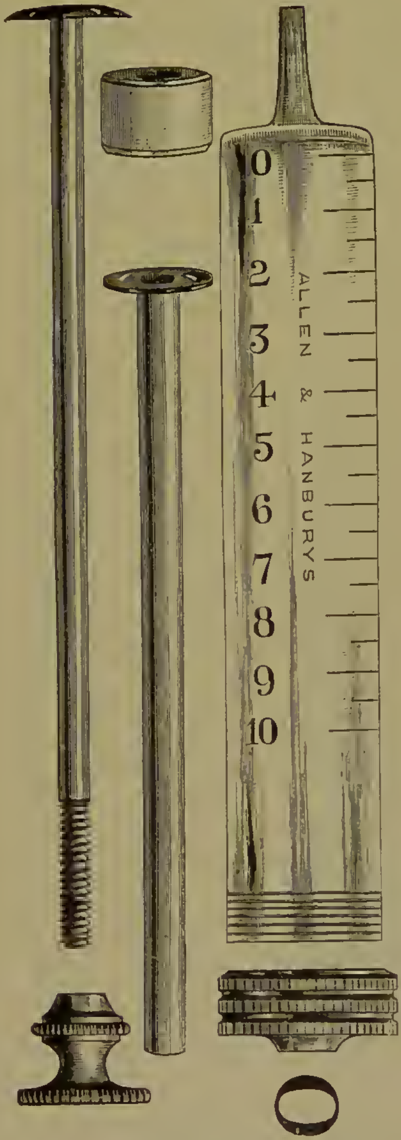

Fig. 16.—Anti-toxin Syringe, showing component parts.

enamelled iron dish heated on a tripod over a bunsen or spirit-lamp flame is best, but in households any small saucepan or kettle may be used. Ordinary tap-water may be used, preferably some that has already been boiled in a kettle in order to ensure sterility, and also to diminish the deposit of salts which takes place when tap-water is boiled. The needle should be detached from the syringe, the syringe filled with the water, the nut on the piston rod loosened, and then the syringe and needle should be boiled for at least five minutes. The syringe is then fitted to the needle, handling the needle only by the socket, the nut on the piston-rod is screwed up so as to obtain a good fit, and the whole, after being rinsed out two or three times with the boiling water, is placed aside to cool, care being taken that the needle touches nothing. The bottle of serum is then taken and the neck may with advantage be wiped round with a pledget of wool soaked in 1–20 carbolic or absolute alcohol to remove any dust that may have

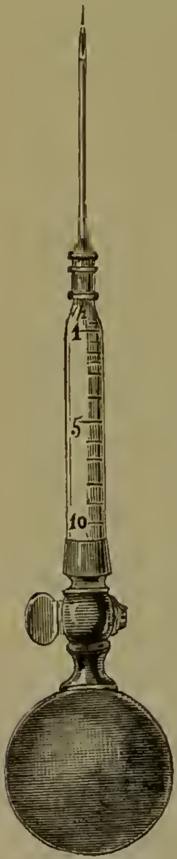

FIG. 17.—KOCH SYRINGE.

collected. If the serum is in a phial which has been sealed, a nick is made with a small triangular file in the neck, so that this may be broken off.

The syringe having cooled may then be filled with the serum, the proper dose as directed in the instructions issued with the particular brand of serum being used. The requisite amount having been sucked up into the syringe, the latter is held upright, needle upwards, and the air expelled, and then the injection may be given.

The preferable seat for the injection is the flank or between the scapulæ. The skin should be disinfected by rubbing with a pledget of wool soaked in 2 per cent. lysol or other efficient antiseptic, and the needle is plunged well into the subcutaneous tissue. No dressing need be applied to the puncture. Not more than 40 c.c. should be given in one place, and when injections are being repeated, they should be administered on alternate sides of the body and in different situations to avoid local irritation as far as possible. In the case of children with whom struggling may be expected, the needle may be attached to the syringe by a short length of rubber tubing (also boiled) to diminish the risk of breakage. In some instances it is advisable to administer the anti-toxin by intravenous injection. This may be done into one of the superficial veins of the forearm or back of the hand. A ligature is placed round the upper arm sufficiently tight to obstruct the venous but not the arterial circulation, so as to render the veins prominent, as in the operation of venesection. The anti-toxin should be warmed by standing the bottle in hot water (40° C. or 105° F.) for a quarter of an hour. The serum should be carefully examined after shaking the bottle to ascertain whether it is turbid or no : if particles are present, it must be strained through some fine muslin which has been boiled for a few minutes into a small cup or other receptacle that has been washed with an antiseptic and afterwards rinsed with some boiling water. The syringe detached from the needle is filled and all air carefully expelled from it. The needle is then inserted obliquely into the vein in the direction of the shoulder, so that its point lies free in the lumen of the vein; when this is the case, the blood drips from it, and the syringe is then attached and the serum slowly injected. There is no danger whatever in this procedure provided the simple precautions detailed above are observed. Intracerebral inoculation, which is employed in tetanus, will be described in the section devoted to tetanus anti-toxin (p. 123).

After use, the syringe should be rinsed out with *cold* water, the needle carefully wiped and a wire inserted in it. The needles may with advantage be kept in a stoppered bottle in absolute alcohol.

Complications and Sequelæ of Anti-toxin Treatment

A. *Complications not peculiar to the treatment. Abscess.*—Abscess at the seat of inoculation is seldom met with, and, with the observance of proper antiseptic or aseptic precautions, should be practically unknown unless the serum has become contaminated. The serum should always be carefully scrutinised before use and any suspicious bottle rejected and returned to the maker. Abscess did not occur in 500 cases treated by Stanley.

Septic infection.—This is almost unknown, and could hardly happen unless the serum were contaminated.

Hæmorrhage.—Slight hæmorrhage sometimes occurs at the seat of inoculation, but does not seem to give rise to any trouble.

Albuminuria.—In diphtheria, albuminuria has been stated to occur as the result of the injection of anti-toxin. As a foreign albumin, such as egg-albumin, is excreted by the kidney on injection, it is possible that anti-toxin (which is a foreign serum) might be similarly excreted. But the general consensus of opinion is that albuminuria is *less* frequent in diphtheria under anti-toxin treatment than formerly. It may be confidently stated that anti-toxin does not give rise to any kidney trouble.

B. *Concomitants and sequelæ peculiar to the treatment.*—There are certain concomitants and sequelæ which are very commonly met with in patients who have received injections of anti-sera ; they are (*a*) various rashes, (*b*) swelling and pain in the joints, and (*c*) pyrexia with or without rash or joint pains. These effects are best known in diphtheria, since the cases of this disease treated with anti-toxin are vastly more numerous than those of any other disease, and the statistics given below refer to it. These effects are solely due to the injection of a foreign serum, such as anti-toxin is, and are not due to the anti-toxic or anti-microbic constituent, and their frequency and severity, therefore, depend to some extent upon the *quantity* of serum injected, and were more often met with in the early days of anti-toxin treatment, when the dose of serum was larger, than at present when the dose is smaller, in consequence of the preparation of more potent sera. But they also depend somewhat upon the source of the serum, and some horses yield a serum which gives rise to these effects to a greater extent than other horses.

That it is the serum and not its anti-toxic constituent which produces these rashes, &c., is proved by the fact that *normal* serum—*i.e.* that from an untreated animal—gives rise to these effects to the same extent as anti-toxic serum, and if anti-toxic serum be heated to 59·5°–60° C. for half an hour, it loses these properties without impairing its anti-toxic value.[1] According to Brodie [2] the rash-producing constituent is soluble in acetone, which does not dissolve the anti-toxin.

In the Report of the Metropolitan Asylums Board upon anti-toxin treatment in 1896 the following statistical table is given of the occurrence of these complications :—

Number of cases treated, 483		
Complications	Number of cases	Percentage of total cases
Rash	183	37·8
Joint pains	55	11·3
Pyrexia, with or without rash or pains .	83	17·1
Abscess at site of injection . . .	6	1·2

In Stanley's series of 500 cases reported in 1902 rashes were met with in 112 cases, or rather more than 20 per cent. Carrière estimates that rashes occur in 14 per cent. of the cases.

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[1] Béclère, *Ann. de l'Inst. Pasteur*, x. 1896, p. 567.

[2] *Journ. of Path. and Bact.* iv. 1897, No. 4, p. 460.

Pyrexia.—This sometimes occurs after anti-toxin treatment, usually between one and three weeks after the commencement, and is generally accompanied by joint affections and rashes.

Rashes.—The appearance of rashes is a very common sequela of anti-toxin injection. The rashes are of varied character, and usually develop some time after the inoculation of the serum, but occasionally early at the seat of inoculation. Stanley gives the following illustrative tables:—

	Cases
Cases of diphtheria receiving anti-toxin	500
Anti-toxin eruptions	112
Erythemata	58
Erythemata + urticaria	15
Urticaria	30
Scarlatiniform	6
Morbilliform	3
Transient early erythema and urticaria (usually at seat of injection)	17

Average day of onset of eruption:—	Day
Erythemata (varied from 4th to 29th day)	12·2
Urticaria (varied from 4th to 19th day)	9·2
All eruptions	10·8

A *polymorphous erythema* is the commonest of all the skin eruptions. It occurs especially upon the extensor surfaces, commencing usually upon the trunk, sometimes on the face, and spreading to the limbs: it may be from vivid scarlet to dusky red in colour, lasts two to three days, may be accompanied by some swelling, and followed by slight desquamation. In size it varies from small spots to large patches, or may cover a large area, and is generally more or less circular in outline. Some general symptoms accompany it—viz. slight malaise, a rise of temperature of 3° F., and sometimes joint pains.

An *urticarial* eruption comes next in frequency, and may sometimes pass into an erythema. It lasts usually about two days.

A *morbilliform erythema* is less frequent than the two preceding and may appear upon the face and trunk. Stanley describes it as being pinker than that of measles, and more nearly resembling rötheln. There may be some rise in temperature and redness of the throat, but no coryza.

A *scarlatiniform erythema* is also not an infrequent eruption, and generally occurs late, with some rise in temperature and general symptoms. It may, in some instances, be difficult to distinguish it from scarlatina, which, not unfrequently, is complicated with diphtheria. The points of distinction are the mildness of the symptoms (neither convulsions nor vomiting, and fever slight), the throat and tongue have not the appearance of scarlatina, and the rash disappears within forty-eight hours.

Stanley states that he has seen a case with a measly rash on the face, a scarlatiniform one on the trunk, and a circinate erythema on the extremities.

Purpuric and *pruriginous* eruptions have also been met with, but are very rare. Transient early erythema and urticaria occasionally appear near the seat of inoculation.

These eruptions do not seem in any way to add to the gravity of the case; they are usually mild and pass rapidly away. Treatment must be on general principles; in the urticaria calcium chloride may be given.

Joint pains.—Swelling and pain in the joints frequently follow the injection of anti-toxin. They usually are of small moment and soon subside.[1]

The Prophylactic Use of Anti-sera

Experimentally, the anti-sera are found to possess immunising properties in a high degree.

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[1] See Stanley, *Brit. Med. Journ.* 1902, i. p. 386; Carrière, *Le Nord Médical*, November 1, 1902, p. 241.

A guinea-pig inoculated with a small amount of diphtheria anti-toxin is thereby rendered insusceptible to many times the fatal dose of diphtheria toxin. This insusceptibility is rapidly acquired—within a few hours—but gradually passes off, so that at the end of a month hardly any trace of it will be left. This immunising property of diphtheria anti-toxin is now being applied practically for prophylactic purposes, and as its value becomes more generally recognised will prove a valuable method for helping to stamp out the disease. Tetanus anti-toxin and the anti-streptococcic and anti-plague sera have also been used as preventives.

The prophylactic use of the anti-sera will be referred to in the sections dealing with each serum.

CHAPTER IV.

THE ANTI-TOXIC SERA

DIPHTHERIA ANTI-TOXIN—TETANUS ANTI-TOXIN—ANTI-VENENE

Diphtheria Anti-toxin

Anti-diphtheritic serum or diphtheria anti-toxin stands foremost among the anti-toxic sera both on account of the great use which is made of it and of the excellent results obtained therefrom. Its mode of preparation is briefly as follows. A virulent diphtheria bacillus is grown in a special broth for ten to fourteen days in the incubator at 37° C.; it is then filtered through a Pasteur-Chamberland or Berkefeld filter to remove the diphtheria bacilli, and the filtrate is employed for injecting the horses, commencing with a dose of $\frac{1}{2}$ to 1 c.c. The injections are given about twice a week, the dose being gradually increased until the serum attains a sufficient degree of potency, as is ascertained by testing the serum in the manner to be described. The treatment will probably extend over a period of five or six months before the horses attain a sufficient anti-toxic power, and the dose of toxin will ultimately reach as much as $\frac{1}{2}$ to 1 litre. At first the injections give rise to considerable constitutional disturbance, with swelling at the seat of inoculation (fig. 18); later, very little disturbance occurs. It is inconvenient to employ a serum the strength of which is below about 400 units per cubic centimetre. The diphtheria bacillus employed must be a highly

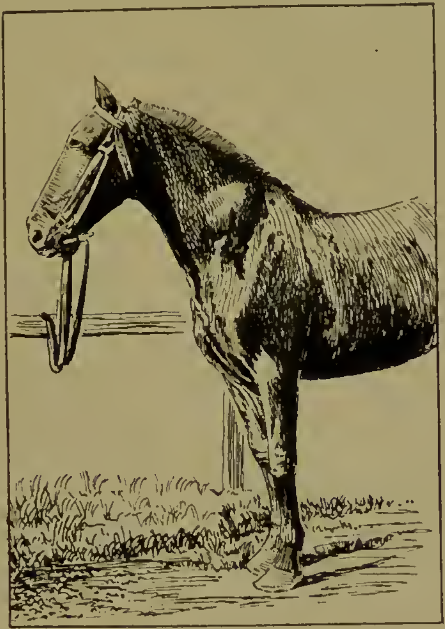

Fig. 18.—Local Swelling at Seat of Inoculation of Toxin during early stages of immunisation for the preparation of Diphtheria Anti-toxin.

virulent one; few strains, in fact, possess the necessary virulence, and most of the diphtheria anti-toxin is obtained by the use of two or three strains, notably one obtained by Behring and a second isolated by Park in America; in the latter case $\frac{1}{200}$, $\frac{1}{400}$, or even $\frac{1}{500}$ of a cubic centimetre of the toxin is sufficient to kill a guinea-pig weighing 250 grams.

The culture-medium may be ordinary beef-peptone-bouillon, alkalised by the addition of 5–7 c.c. of normal caustic soda solution per litre after neutralisation. Spronck has recommended the use of meat which has been kept for some days until incipient putrefaction has taken place in order to destroy the muscle sugar which he believes interferes with the toxin production. Formerly the bacillus was grown for four or five weeks in special flasks, so arranged that a current of air could be continuously aspirated through the culture; but this method has now been given up, the medium being distributed in ordinary Erlenmeyer flasks each containing about half a litre, and being grown for about eight to twelve days. It is important to obtain a growth upon the *surface* of the medium. With ordinary strains of diphtheria bacilli this rarely occurs spontaneously; in order to obtain it, a serum culture may be rubbed up with some sterilised cork raspings, which are then added to the flasks of culture-medium. The cork particles with attached bacilli float, and from them a growth starts and spreads over the surface. Once having obtained the surface growth, it is transferred to the fresh flasks of culture-medium. The American bacillus shows a great tendency to produce this surface growth.

L. Martin prepares the bouillon for the diphtheria toxin from the stomach of the pig. The pig's stomach (muscular and mucous coats) is minced fine, and of this 200 grams are placed in 1,000 c.c. of water acidified with 10 c.c. of pure hydrochloric acid. The mixture is kept at 50° C. for twelve to twenty-four hours, then heated to 100° C. for a few minutes, filtered through cotton-wool, heated again to 80° C., and rendered alkaline ; filtered through paper, heated again to 120° C. in the autoclave, filtered once more through paper, and filled into flasks, which are then sterilised by heating to 115° C. for fifteen minutes. This method is still employed at the Pasteur Institute. Spronck has also devised a yeast-water medium for the cultivation of the organism.

Cartwright Wood has devised a method for immunising the horses by which he claims that the period of treatment may be much shortened.

It consists in cultivating the diphtheria bacillus in ordinary broth, to which has been added ten to twenty per cent. of blood-serum, at a temperature of 37° C. for at least three or four weeks; for use this is heated to 65° C. for an hour and then filtered through a Pasteur-Chamberland filter. Of this heated serum-toxin two or three injections of 100 to 200 c.c. are administered at intervals of some days, after which the serum-toxin plus ordinary toxin, or ordinary toxin alone, is given. The effect of the serum-toxin seems to be to produce a rapid immunity, so that considerable doses of ordinary toxin can be soon given and the tedious preliminary stage of the treatment much shortened. More recently he has found that the serum used for the culture-medium must be homologous with the animal that is to be treated—*i.e.*, if the horse is to be treated, horse serum must be employed; if the rabbit, rabbit serum, and so on. Other sera, such as ox or sheep, added to the culture-medium, had for the horse little or no effect in producing rapid immunity.

In the earlier stages of injection the dose must be cautiously increased; all reaction caused by the previous dose being allowed to subside completely before another one is administered. Some horses are decidedly more susceptible than others, and, generally speaking, probably a susceptible horse is likely to yield a more potent anti-toxin than one which is not so susceptible. Certain horses cannot be made to yield a potent anti-toxic serum; no matter how large the doses of toxin, and for how long the treatment is prolonged, their serum does not acquire a high anti-toxic value, although their immunity to the toxin may be very marked. It is impossible to say upon what factors these differences depend.

The best times for injecting the toxin and for bleeding the animal are of some importance and have been elucidated through the work of Salomonsen and Madsen. These observers found that on injecting a large volume of toxin a fall in the anti-toxic content of the blood takes place. This, however, is soon recovered from; the anti-toxic content begins to rise again about the third day, and reaches a maximum about the ninth or tenth day, being then higher than before the injection. The proper time for bleeding the animal is therefore about the tenth day after the last injection. With regard to the best time for repeating the injections of the toxin, Dean believes this to be on the third day after the last injection. The fact that an animal, after yielding anti-toxin for a shorter or longer period, becomes exhausted as it were, and its serum progressively diminishes in anti-toxic value, has been referred to above (p. 44). This generally occurs with the animals yielding diphtheria anti-toxin.

LITERATURE

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The Standardisation of Diphtheria Anti-toxin

Various methods have been adopted for the standardisation of diphtheria anti-toxin. In Roux's method the lethal dose of the toxin having been ascertained for a 450 to 500 gram guinea-pig, the number of grams of guinea-pig which can be immunised by 1 c.c. of anti-toxin is calculated. For example, if 0·01 c.c. of anti-toxin will neutralise the lethal dose of toxin when injected simultaneously into a 500 gram guinea-pig, the immunising value of the serum is said to be 50,000 (=500 × 100). But this method has many objections; in using a single lethal dose of toxin, it is evident that if only a small fraction of the toxin be neutralised death will not ensue, and therefore the method may fail to give the actual immunising value of the anti-toxin. Thus, in the above example it is quite possible that only three-quarters of the minimal lethal dose might be neutralised, and therefore the true immunising value of the serum would be not 50,000, but $\frac{3}{4} \times 50{,}000 = 37{,}500$—a very great difference. It is therefore preferable and customary where Roux's method is employed to work with a multiple of the minimal lethal dose, generally ten lethal doses (Behring's standard), as in the case of tetanus anti-toxin, and of anti-venene; but for diphtheria anti-toxin a far more accurate method has been devised by Ehrlich.

The strength of the diphtheria anti-toxin is estimated in 'units,' the 'unit' being an arbitrary standard. It was arrived at originally by mixing ten times the minimal lethal dose of a diphtheria toxin with varying amounts of the anti-toxin and injecting subcutaneously into a guinea-pig of 250 grams weight and ascertaining the quantity of serum which was just necessary to neutralise this dose of toxin. This amount of serum was said to contain $\frac{1}{10}$ of an anti-toxic unit, and one unit was therefore the equivalent of 100 lethal doses of toxin.

The unit refers to the *strength* or the neutralising power of the serum towards the toxin, and not to the *amount* of the serum; in fact, one unit may be contained in 0·1 c.c., 0·01 c.c., 0·005 c.c., or even less of the serum. In this method of testing it was taken for granted that 100 minimal fatal doses of any toxin would be neutralised by one unit of anti-toxin. But in 1896 Ehrlich in a profound study of the diphtheria toxin found that this was not the case, and that the same amount of anti-toxin might neutralise anything from 16 to 136 minimal fatal doses of various toxins (see T. Smith, *Journ. Boston Soc. of Med. Science*, vol. v. No. 1, 1900, p. 1).

In order to explain this it will be necessary to consider briefly the constitution of the diphtheria toxin broth.

Constitution of diphtheria toxin.—The constitution of diphtheria toxin is much more complex than was at one time supposed. If in the case of three toxins the minimal lethal dose for a guinea-pig is 0·5 c.c. for the first, .0·03 c.c. for the second, and 0·002 c.c. for the third, and if the toxin broth in each case is a solution of a pure substance, the toxin, a body characterised by the same toxic and combining properties with anti-toxin, it follows that these three toxin broths are merely solutions of toxin of varying strength, and that therefore the same amount of toxin must be present in each lethal dose, that is one 'lethal guinea-pig toxin equivalent.' On this assumption the same amount of anti-toxin ought to render the lethal doses of each of the three toxin broths innocuous, but such is not the case. It is found with various toxin broths that very different amounts of the toxin are neutralised by a uniform amount of anti-toxin, an amount which may be termed 'one immunising unit.' Ehrlich has found that the L_0 dose of various toxins—that is, the amount of toxin which is exactly neutralised by 1 immunising unit of anti-toxin (see p. 12)—varied from 16 to 136 lethal guinea-pig doses; moreover a toxin broth in course of time gradually diminishes in toxicity, but at the same time there may be no alteration in its neutralising power for anti-toxin; that is, the L_0 dose of the fresh toxin may be exactly the same as that of the old toxin. It follows from this that there are present in the toxin broth substances which, though non-toxic, are capable of combining with anti-toxin: to these substances the names of toxoids and toxones are given. The toxoids are bodies which are formed by alterations in the toxin, the toxones are primary secretory products of the diphtheria bacillus, formed together with toxin during its growth. The toxones (formerly termed epitoxoids) possess less affinity for the anti-toxin than toxoids and toxins, so that in the course of the partial neutralisation of toxin broth with anti-toxin, the order of combination with anti-toxin is first the toxoids, then the toxin, and lastly the toxones. There are also at least three species of toxoid: proto-, deutero-, and trito-toxoid: of which proto-toxoid has the greatest affinity for anti-toxin, becoming neutralised by it before anything else. The toxoids seem to be comparatively innocuous, but the toxones, while not possessing any acute toxic action, have the power of producing a certain amount of induration at the seat of inoculation, but not in so marked a degree as toxin, and of inducing the slowly developing diphtheritic paralyses. Since by neutralisation is meant that there are not only no general symptoms of intoxication but also no local ones, such as swelling and induration at the seat of inoculation, all these constituents have to be taken into account in the process of standardisation.

Process of standardisation.—Anti-toxin has been made the standard for testing purposes, and not toxin, on account of its better keeping qualities. Standard anti-toxin is supplied by the 'Serumprüfungs-Institut,' Frankfort-on-Maine. It is dried over phosphoric anhydride and is kept *in vacuo* in sealed tubes. Each tube contains two grams of the dried anti-toxin, equivalent to 1,700 units in each gram.

Standard Anti-toxin Solution.—With the standard anti-toxin the laboratory toxin is first standardised, and then with this standardised toxin any anti-toxin may in its turn be standardised. The anti-toxin is dissolved in 200 c.c. of a solution consisting of equal parts of glycerin and 10 per cent. sodium chloride solution. Each cubic centimetre of this solution contains therefore 17 units of anti-toxin; this forms the *stock* solution, and it retains its potency unimpaired for three months at least, if kept in a cool and dark place. Solutions of anti-toxin are also sent out by the 'Serumprüfungs-Institut' for testing purposes; their strength varies and is marked on the bottle. For use 1 c.c. of the stock solution is measured out and mixed with 16 c.c. of tap-water, or with any other solution; it is diluted in such a manner that 1 c.c. contains one unit. This forms the *test* solution and contains one unit in every cubic centimetre. For measuring out the solution of anti-toxin, a pipette graduated for *glycerin* should be used, or, failing this, it must be run out slowly from a 1 c.c. pipette and a single drop in excess of 1 c.c. allowed to compensate for the fluid which clings to the glass of the pipette. The operations must be carried out with the greatest care and delicacy, for the process is comparable to a volumetric chemical analysis.

The Test Toxin.—The toxin broth should be an active one. It is filtered through a porous porcelain filter, and the filtered toxin broth or 'toxin' is preserved by the addition of some pure toluol which should form a layer a quarter of an inch in thickness on the surface of the toxin. The toxin must be kept in the dark in a cool place, preferably upon ice, and in large bulk, and has to be re-standardised about every month.

Standardising the Toxin.—First of all the minimal lethal dose of the toxin is ascertained approximately by injecting various amounts into guinea-pigs weighing 250 grams. In order to attain greater accuracy, if the toxin is an active one it should be diluted ten to one hundred times with tap-water, so that errors introduced by the measurement of small quantities may be reduced to a minimum.

The smallest amount of toxin which will kill with certainty on the fourth or fifth day after injection of the toxin is the minimal lethal dose. For testing purposes there is no need to ascertain the minimal lethal dose with great accuracy. Ehrlich's normal diphtheria toxin

(DTN 1) is one in which the minimal lethal dose is 0·01 c.c., a toxin in which the minimal lethal dose is 0·005 c.c. is DTN 2, one in which it is 0·02 is DTN ·5, and so on.

The following table (Table I.) illustrates the method of testing.

TABLE I.—THE MINIMAL LETHAL DOSE OF A TEST TOXIN

Dose of Toxin	Weight of guinea-pig in grams					
	—	24 hours	48 hours	72 hours	Fourth day	Fifth day
0·01 c.c. .	275	235 *l*	dead			
0·005 c.c. .	265	233 *l*	200	dead		
0·0033 c.c. .	263	230 *l*	230	235	220	dead
0·0025 c.c. .	260	235 *l*	215	225	210	died on ninth day

l = local reaction.

From this testing therefore the acute lethal dose is about 0·003 c.c.

Next the maximum amount of the toxin which is just neutralised by one unit of standard anti-toxin, and the amount of toxin which when mixed with one unit of standard anti-toxin is unneutralised to the extent that death is caused on the fourth or fifth day, are ascertained by injecting varying amounts of toxin mixed with one unit of standard anti-toxin into guinea-pigs. The former, the maximum amount of toxin which is just neutralised by one unit of standard anti-toxin, is termed the L_0 dose and need be ascertained only approximately; the latter, the amount of toxin which when mixed with one unit of standard anti-toxin causes death on the fourth or fifth day, is termed the L_+ dose, is the more important, and must be estimated with the greatest accuracy. In standardising, only the L_+ dose is used.

The method of ascertaining the L_0 and the L_+ doses is as follows. Into each of several small conical glasses, termed test-glasses, 1 c.c. of the test solution of the standard anti-toxin is measured, *i.e.* one unit, and with this are mixed different amounts of toxin varying from a little below up to considerably above one hundred times the minimal lethal dose of toxin. Each mixture is made up to a volume of about 4 c.c. with tap-water, and is injected subcutaneously into a guinea-pig. The guinea-pigs used for all the processes of testing must weigh as nearly 250 grams as possible, the lighter weights being used for the mixtures containing the smaller amounts of toxin, and the animals must be kept as far as possible under the same conditions. The test-glasses hold about 5 c.c., are made from glass tubing of $\frac{1}{2}$ inch internal diameter, and are 1 inch deep, the upper $\frac{1}{2}$ inch being cylindrical, the lower $\frac{1}{2}$ inch conical and tapering to a point. They are supported in a stand consisting of a block of wood drilled with suitable holes. The object of the conical shape is to ensure the whole of the mixture being drawn up into the syringe, and the mixture is diluted with tap-water in order that the loss of toxin caused by the small amount that adheres to the glass may be reduced to a minimum. The mixture must be injected, by means of a sterile 5 c.c. syringe, entirely subcutaneously. The animals are weighed at the time of injection and daily afterwards at about the same hour. If the toxin is unneutralised and is exerting its toxic action, the weight of the animal steadily falls; the more acute the intoxication, the more rapidly the weight falls. The seat of inoculation must also be examined daily; when the toxin is not completely neutralised, more or less local reaction is present in the form of swelling and induration and sometimes even of necrosis. In this manner, first of all the L_+ dose of the toxin is found between two limits, and when this has been done a further series of experiments have to be performed with amounts between these limits in order to obtain the exact amount correct to the second decimal place. Coincidently, the amount of the L_0 dose will be approximately ascertained; it is required to be known only to exclude an unsuitable toxin (see below. The following tables (Tables II. and III.) illustrate the method of testing.

TABLE II.—TESTS MADE TO ASCERTAIN THE L_+ DOSE OF A TOXIN

The minimal lethal dose of the toxin was 0·002 *c.c.*

Amount of the Toxin mixed with one unit of Anti-Toxin	Weight of guinea-pigs in grams					
	—	24 hours	48 hours	72 hours	96 hours	120 hours
0·1 c c. . .	248	245	250	253	260	268
0·2 c.c. . .	250	245	259	255	270	278
0 3 c.c. . .	250	225	dead			
0·4 c.c. . .	255	dead				
0·5 c.c. .	255	dead				

From this series of experiments the L_+ dose of this toxin lies between 0·2 and 0·3 c.c.; 0·2 c.c. is too little and 0·3 c.c. is too much, and further experiments have to be performed in the same manner to ascertain the exact amount to the second decimal place. The L_0 dose is very nearly 0·2 c.c.

TABLE III.—THE L_+ DOSE OF THE TOXIN (*continued*)

The L_+ dose from Table II. lies between 0·2 *and* 0·3 *c.c.*

Amount of the Toxin mixed with one unit of Anti-toxin	Weight of guinea-pigs in grams				
	—	24 hours	48 hours	72 hours	—
0·24 c.c. . .	273	256 *t*	277 *s*	285	lived
0·26 c.c. . .	230	195 *t*	210 *sl*	220	lived
0·28 c.c. . .	255	228 *sl*	236 *sl*	242	lived
0·30 c.c. . .	248	243	dead		
0·31 c.c. . .	250	248	dead		

t = trace of, and *sl* = slight, local reaction.

From this second series of experiments it follows that the L_+ dose of the toxin lies between 0·28 and 0·3 c.c., and further experiments have to be performed to arrive at the exact amount. The standardisation of a test toxin may thus entail the sacrifice of a large number of animals, but this is unavoidable.

In a satisfactory toxin for testing purposes the L_+ dose should not exceed 1 c.c., and the difference between the L_+ dose and the L_0 dose ($L_+ - L_0$) should not exceed about 15 minimal lethal doses. If toxin broth contained toxin only, and no other substances which have an affinity for anti-toxin, the difference between the L_+ dose and the L_0 dose would be equal to the minimal lethal dose ($L_+ - L_0 = 1$ MLD); but since toxoids, which have a greater affinity for anti-toxin than toxin has, are always present in addition, and combine first with the anti-toxin and, as it were, use up a portion of this, the difference between the two is always a multiple of the minimal lethal dose ($L_+ - L_0 = x$MLD). The L_+ dose therefore usually corresponds to about 110–115 minimal lethal doses, but Ehrlich has found that it varies from 16 to 136 minimal lethal doses as extremes.

If toxin broth contained toxin only and no toxoids and toxones, the L_0 dose and the L_+ dose would be 200 and 201 minimal lethal doses respectively. The standard unit of anti-toxin can now be defined: it is that amount of anti-toxin which would exactly neutralise 200 minimal lethal doses of a hypothetical toxin containing toxin only. But, inasmuch as such a toxin has not yet been prepared, it corresponds roughly to 100 minimal lethal doses of the ordinary toxin. For ordinary purposes the unit of diphtheria anti-toxin may be defined as that amount of anti-toxin which will neutralise about 100 lethal guinea-pig doses of diphtheria toxin.

Standardisation of Anti-toxin.—A suitable diphtheria toxin having been prepared and having been standardised with standard diphtheria anti-toxin as described above, and the L_+ dose having been ascertained with accuracy, this standardised toxin in its turn is employed to standardise any diphtheria anti-toxin that may have been prepared. The method of procedure is as follows. First the anti-toxic serum to be tested must be considerably diluted; if it is believed to contain at least 100 units per c.c., as is usually the case, it is diluted one hundredfold, if less fifty-, twenty-, or tenfold. In the first case, one cubic centimetre of the serum is accurately measured out by means of a 1 c.c. pipette and run into a 150 c.c. flask; 99 c.c. of tap-water are then added from a 100 c.c. burette, the water being run slowly out of the burette so as to allow time for the water that adheres to the glass to run down. The water and serum are thoroughly mixed, but, to avoid air-bubbles, should not be shaken, and the mixture may with advantage be allowed to stand for half an hour to allow the froth to subside. The serum is thus diluted so as to form a one per cent. solution. Varying amounts of this diluted serum are then placed in the test-glasses; to each is added the L_+ dose of the standardised toxin, the mixture is made up to about 4 c.c. with tap-water, and the whole is injected subcutaneously into a 250 gram guinea pig.

The following example will render this description clearer.

TABLE IV.—TESTING THE ANTI-TOXIN

An Anti-toxin to be tested for 100, 200, 300, 400, *and* 500 *units per c.c.*

Of the dilute solution of anti-toxin (1 c.c. = 0·01 c.c. of anti-toxin), 1 c.c., 0·5 c.c., 0·33 c c., 0·25 c.c., and 0·20 c c. are placed in five test glasses respectively, to each is added the L_+ dose of the test toxin (in this instance 0·4 c.c.), and each is made up approximately to 4 c c. by the addition of tap-water. Each mixture is then injected into a guinea-pig.

Number of units tested for	Weight of guinea-pigs in grams				
	—	24 hours	48 hours	72 hours	Fifth day
100	250	260	260	268	280
200	250	293 *t*	305 *t*	310 *t*	320 *t*
300	255	225 *sl*	275 *sl*	295 *t*	305 *t*
400	260	290 *l*	275 *l*	268 *l*	232 *n*
500	265	273	dead		

t = trace of, *sl* = slight, *l* = large, local reaction, *n* = necrosis at seat of inoculation.

From these experiments it follows that this specimen of anti-toxin contained about 300 units per cubic centimetre.

This, then, is the method by which diphtheria anti-toxin is tested and standardised, and it will be seen that it is by no means an easy one and can only be carried out by an expert.

LITERATURE

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Therapeutic Use of Diphtheria Anti-toxin

Value of Anti-toxin.—The immense value of diphtheria anti-toxin in the treatment of diphtheria cannot be doubted by any unbiassed observer. In the hospitals of the Metropolitan Asylums Board there has been a progressive fall in the case-mortality since the introduction of anti-toxic treatment, as the following table (p. 95) shows (*Metrop. Asylums Board Rep.* 1901).

The statistics published by the Chicago Health Department, although two years old, are so striking as to be worthy of attention. During the five years preceding anti-toxin treatment

Cases of Diphtheria in the Hospitals of the Metropolitan Asylums Board

Year	No. of cases admitted	Deaths	Mortality per cent. of patients treated	Annual mortality per 1,000 of estimated population
1888	99	46	59·35	0·32
1889	722	275	40·74	0·39
1890	942	316	33·55	0·33
1891	1,312	397	30·63	0·32
1892	2,009	583	29·35	0·46
1893	2,848	865	30·42	0·76
1894*	3,666	1,035	29·29	0·62
1895	3,635	820	22·85	0·54
1896	4,508	948	21·20	0·60
1897	5,673	987	17·69	0·51
1898	6,566	991	15·37	0·39
1899	8,676	1,182	13·95	0·43
1900	7,873	988	12·27	0·34
1901	7,622	849	11·15	0·29

* 1894, the year in which anti-toxic treatment was commenced.

there had been an aggregate of 7,411 deaths from diphtheria and croup, an annual average of 1,482 deaths and an annual death rate of 11·23 per 10,000 of population. Anti-toxin treatment was commenced on October 5, 1895, and during the five years ending December 31, 1900, there was an aggregate of 4,309 deaths from diphtheria and croup, giving an annual average of 862 deaths and an annual mortality rate of 5·45 per 10,000 of population. These figures show a reduction upon the five-year pre-anti-toxin period of nearly forty-two (41·96) per cent. in the actual numbers and of nearly fifty-two (51·72) per cent. in the mortality rate. The case-mortality of diagnosed diphtheria during the years 1891–1895 averaged about 35 per cent. During the 63 consecutive months between October 1895 and December 1900 the case-mortality rate was less than seven (6·79) per cent.—a reduction of 80 per cent. upon the pre-anti-toxin rate.

As far as possible the inspectors of the Health Department visit every case of sore throat; if it be suspicious, anti-toxin is used without waiting for a bacteriological examination; hence a large proportion of the cases are treated within the first day or two of the disease. Moreover, every case of 'sore throat' is examined bacteriologically.

The record of 5,727 cases shows two deaths in 476 treated on the first day of the disease—a mortality rate of 0·42 per cent.; 22 deaths in 1,426 cases first treated on the second day—a mortality rate of 1·54 per cent.; 73 deaths in 2,034 cases first treated on the third day—a mortality rate of 3·59 per cent.; while there were 118 deaths in 1,037 cases first treated on the fourth day of the disease—a mortality rate of 11·38 per cent.; and 174 deaths in 754 cases treated later than the fourth day, or over 23 per cent. These figures show strikingly the value and importance of early treatment, which are still more manifest from the following considerations. In 3,936 cases of diphtheria treated with full doses of anti-toxin on the third day of the disease or earlier, there were 97 deaths—a mortality rate of less than *two and a half* (2·46) per cent. In the remaining 1,491 cases first treated on the fourth day or later there were 292 deaths—a mortality of more than 19·5 per cent.

For these statistics, diphtheria and all croup, including catarrhal, cases are grouped together to eliminate error that might otherwise be caused by changes in diagnosis and nomenclature. Estimated on the money basis of the value of a human life, the saving of life during the anti-toxin period in 1900 represented a saving of nearly a million and a half pounds! Two other interesting and complete summaries of the influence of anti-toxin in reducing the death rate from diphtheria in a number of large towns, &c., are given by Cobbett and by Rosenthal.

With regard to the clinical use of anti-toxin in diphtheria, Burrows has published an elaborate study based upon 2,093 cases treated by him with anti-toxin in the Boston City Hospital. Of this number 131 proved to have a mixed infection and 1,962 were uncomplicated. Of the uncomplicated cases 240 died, giving a death-rate of 12·23 per cent. But of the 240 fatal cases, 69 were moribund and died within twenty-four hours of admission, and if these be deducted the death rate so modified would be 9 per cent. The 131 cases of mixed infection were coincident scarlatina and diphtheria.

Goodall gives an exhaustive report upon the value of anti-toxin in the treatment of diphtheria and shows how the case-mortality has fallen under the treatment. In cases treated with anti-toxin, the extension of existing and formation of fresh membrane are stopped, and that already present clears off rapidly. When the nasal passages are affected, the foul discharge that is so often present quickly ceases, greatly to the comfort of the patient. The lessening of the faucial inflammation allows the patient to breathe and to take nourishment without discomfort. The enlargement of the cervical glands and the inflammation of the cellular tissues of the neck subside. There is a visible improvement in the condition of the patient, pulse-rate and temperature fall, appetite returns and convalescence is soon established.

But the value of anti-toxin is perhaps most strikingly seen in laryngeal cases. From the large mass of statistics collected by Goodall and others, it will be found that about 33 per cent.

only, of all laryngeal cases, whether operated upon or not, recovered before the introduction of anti-toxin ; while of the cases not operated upon about 47 per cent., and of tracheotomy cases not more than about 29 per cent., recovered. Since the use of anti-toxin, of all laryngeal cases about 72 per cent. recover, while of the cases not operated upon about 80 per cent., and of the tracheotomy cases about 63 per cent., recover.

Among the 1,962 cases of diphtheria studied by Burrows, there were 337 cases with laryngeal stenosis. Of these, 213 were intubed, but the remaining 124 responded promptly to the use of anti-toxin and were relieved without the necessity for intubation. Of the 213 intubations, 96 died ; reintubation was necessary in many cases, in one as many as thirteen times. Of the intubations a subsequent tracheotomy was required in three. Burrows remarks that the experience gained in these cases ‘ leads to the overwhelming conviction that primary tracheotomy no longer has a place in the treatment of simple diphtheritic laryngeal stenosis.’ He concludes his valuable paper with the remark that ‘ there can be no disease more fascinating than such a one over which science and the skill of the clinician have so nearly gained complete control ’ (through anti-toxin).

Shurley also believes in intubation rather than tracheotomy and has reported 200 cases treated with intubation and anti-toxin with 149 recoveries. He advocates the free and early use of anti-toxin.

It is to be remarked that the American physicians are greatly in favour of intubation rather than tracheotomy. Tracheotomy is doubtless a much easier procedure for those who have no experience of intubation, but when the experience can be obtained, intubation would seem to be worthy of a more extended trial than appears to have been given it in this country.

With regard to the incidence of paralysis, this seems to have increased slightly since the introduction of anti-toxin treatment (see Woollacott). The explanation given to account for this is that more cases, and especially the bad ones, recover now than formerly, and consequently more cases of paralysis are met with, and this is doubtless correct. Ransom has made an *experimental* study of the occurrence of paralysis after the injection of toxin and anti-toxin, and formulates the following conclusions :

1. Paralysis may certainly be expected after intoxication with not less than one-fourth of the

minimal fatal dose of toxin ; it may occur with doses between one-fourth and one-eighth, but not when the dose is below one-eighth.

2. Anti-toxin given fifteen to twenty-two hours after intoxication, with doses not greater than the lethal dose, exercises in large doses a mollifying influence on the subsequent paralysis. Small doses of anti-toxin have no evident effect in diminishing the paralysis.

3. Transferring these results to practice among human beings, we may expect liberal doses of anti-toxin, given early in the illness, to influence favourably the subsequent paralysis, and this beneficial influence is likely to manifest itself not so much on the local paralyses (soft palate, &c.), as on such symptoms as failure of the heart. Severe cases are, however, likely to be followed by some paralysis in spite of even large doses of anti-toxin.

Dosage.—The amount of anti-toxin to be administered has been much discussed. In the early days of the treatment, the doses given were undoubtedly too small. Woollacott considers that 4,000 units should be the ordinary minimum dose. Anderson thinks that a single dose of 4,000 units may suffice for a mild case ; in severe cases he recommends 4,000 units every three hours for three or four doses, repeated the next day if it appears necessary. He prefers to give 4,000 units repeated frequently rather than 8,000 –12,000 units at a single injection, and remarks that the appearance of the membrane is the chief criterion determining the repetition of the dose. If nasal discharge is present, and has entirely ceased, the injections may be discontinued. If no nasal discharge is present, but the membrane appears sodden and has a well-marked loosened edge, not much more anti-toxin will be required. The course of the temperature is not much guide, though there is generally a rapid fall in uncomplicated cases.

Burrows points out that there is unfortunately no way of estimating the amount of toxin that may have been absorbed by a given patient; the amount of membrane is an uncertain guide, and the number of bacilli, their virulence, and the susceptibility of the patient are unknown quantities. He gives as a rule 4,000 units, which dose is repeated every four hours as long as may be necessary. In some bad cases 4,000 units were given every two hours, and in others 8,000 units every four hours for a time. Beggs states that in practice the dose will probably range from a minimum of 3,000 units to a maximum of 24,000 units. To sum up, the dose should probably range from 3,000–4,000 units to 8,000 units. If more be given, it would be preferable in my opinion to administer in smaller amounts at short intervals rather than in a single massive dose. The injections should be repeated until the disease has manifestly subsided.

Early treatment is of paramount importance; in cases treated within the first twenty-four hours or so the mortality is practically *nil.* Every hour's delay lessens the chance of successful treatment. In any case of suspected diphtheria, *anti-toxin should be given at once* without waiting for the result of the bacteriological diagnosis.

No matter how mild the case may seem to be, if there is a reasonable suspicion that the disease is diphtheritic, or if the bacteriological examination shows the presence of diphtheria bacilli in an otherwise unsuspected case, a single dose of 3,000–4,000 units should be given: it can do no harm.

In a case coming under observation late, *i.e.* after the second or third day, the first dose of anti-toxin may with advantage be administered intravenously. Valuable time is thereby saved (Cairns). Anti-toxin should not exclude ordinary treatment. A chlorine gargle or spray is useful, but should not be used if there is struggling. Careful feeding must be the rule. Burrows remarks that digitalis should be used with caution, if at all, on account of the weakened state of the cardiac muscles. Hot-packs may be used with great benefit when the urinary secretion is diminished, and drachm doses of a saturated solution of magnesium sulphate given every hour prove a valuable diuretic in children. Vomiting may be treated with rectal feeding. The recumbent position must be absolutely maintained, and the greatest caution is required in allowing patients to sit up. The heart is usually a reliable guide, and if not affected by a short time out of bed, the time up each day may be gradually lengthened. Patients should sit quietly in a chair for fifteen minutes on the first day out of bed, and they are not allowed to walk to and from the chair.

Albuminuria, so far from being increased by anti-toxin, is often not met with in those patients treated early and with large doses of anti-toxin; water, lemonade, and magnesium sulphate were the only diuretics employed by Burrows.

In the foul sloughing throats sometimes met with in diphtheria the streptococcus seems to be associated with the diphtheria bacillus, and it has therefore been suggested that injections of anti-streptococcic serum should be given in addition to diphtheria anti-toxin. This combined treatment has not however found much favour, but it might prove useful in certain instances and should be borne in mind.

Diphtheria anti-toxin has also been used in conditions other than diphtheria (see p. 158).

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The Prophylactic Use of Diphtheria Anti-toxin

The prophylactic use of diphtheria anti-toxin is especially indicated where a case or cases of diphtheria occur among susceptible individuals who are more or less closely associated, as for example in families, schools, and institutions. Under such conditions, as soon as the primary case is recognised, all those who in any way may have come in contact with it, or better still all the susceptible individuals in the institution, should without delay be injected with a dose of diphtheria anti-toxin. Many records have now been published as to the efficacy of prophylactic injections as a preventive. Biggs, the Director of the New York Health Department, states that of 3,100 individuals known to have been exposed to the infection of diphtheria, and injected with a prophylactic dose of anti-toxin, only 9 contracted the disease, and these in a mild form. The immunising dose was however too small, 150 units, and we give more than this now. The Department of Health of the City of New York, with its usual energy, has issued a 'circular to physicians setting forth the importance of immunisation for the prevention of diphtheria:'

'From January 1, 1895, to January 1, 1900, immunising injections of anti-toxin were administered to 6,806 individuals by the Inspectors of the Department of Health. Of these individuals 18 contracted diphtheria of a mild type; one case only, of diphtheria complicated with scarlet fever, terminated fatally. It is probable that in these 19 cases an insufficient amount of anti-toxin was used to produce immunisation.

'The records of the Division of Bacteriology show that from January 1, 1898, to January 1, 1900, 682 cases of diphtheria occurred which were secondary to an original case in the same family. Under "Secondary" are included only those cases which occurred at least twenty-four hours after and within thirty days of the primary case. Of these 682 cases, 61 died, a mortality of 8·9 per cent. Had these 682 individuals received anti-toxin when the physician first visited the family, probably not one of them would have contracted the disease. The above figures represent only a fraction of such secondary cases occurring in New York City during 1899.

'The Board of Health strongly advocates anti-toxin immunisation in diphtheria. Physicians are especially urged to immunise every child under their care who has been exposed to infection from a case of diphtheria. If this be done, it is believed that the number of cases of diphtheria occurring in the city will greatly diminish. To this end the Department of Health offers to furnish anti-toxin for immunising purposes free of charge. When the physician so desires, the anti-toxin will be administered by the Inspectors of the Department of Health.'

The Health Department of Burton-on-Trent has also issued a circular to the medical profession of the district, emphasising the value of anti-toxin as a prophylactic. In England several examples have been published of the prophylactic value of anti-toxin.

Porter gives some interesting details of an epidemic which occurred in the combined rural districts of Chelmsford and Maldon. There were

24 families in which cases of diphtheria had occurred. The remaining unaffected members of these families comprised 144 individuals, and to 136 of these prophylactic injections of diphtheria anti-toxin were given, and among them a single doubtful case of diphtheria occurred. Of the 8 uninjected individuals, 3 subsequently developed diphtheria. In another series of 24 families, no member of which was injected, of 125 individuals, 21 subsequently developed diphtheria.

In a convalescent home containing 38 children, three consecutive cases of diphtheria occurred. The remaining 35 children were each injected with diphtheria anti-toxin (334 units each), and no further case developed (P. B. Blake).

A serious outbreak of diphtheria occurred in the districts of Cambridge and Chesterton in the autumn of 1900, but by energetic measures of isolation and prophylactic injection the epidemic was stamped out (L. Cobbett).

Jump discusses the immunity produced by the injection of diphtheria anti-toxin for prophylactic purposes, and quotes the views of various authors. He considers that infants may have a longer artificial immunity, just as they are less susceptible to the disease. His own practice has been to isolate the sick child, disinfect the rooms he has occupied, and remove the unaffected children from the house when possible, at the same time giving an immunising dose. The amounts used by various observers for this purpose vary from 100 to 500 units. Jump concludes :

1. That as diphtheria anti-toxin is practically harmless, all exposed persons should receive an immunising dose in proportion to age.

2. That 250 units should be given to children under two years, and 500 to all others.

3. That the immunity will last for at least three weeks, provided a reliable anti-toxin be used.

4. That all exposed persons should be removed from infected surroundings, either by thorough disinfection of their own quarters or by removal to other places. If this be impossible, the immunising doses should be repeated every third week.

With regard to the amount of diphtheria anti-toxin required for prophylactic purposes, probably *as a minimum*, 300 units should be given to children and 500 units to adults : it would be better to administer in all cases at least 500 units. Although the immunity induced by the injection is rapidly acquired, probably within a few hours, it slowly passes off, and cannot be regarded as lasting for more than three weeks.

LITERATURE

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Tetanus Anti-toxin

The writer was the first in this country to prepare tetanus anti-toxin, the most potent of the anti-toxins, by immunising a horse with tetanus toxin. It had previously been prepared by Roux and Vaillard and by Tizzoni and Cattani. The method of immunisation employed is similar to that already described for diphtheria anti-toxin, the horse being injected with increasing doses of tetanus toxin instead of diphtheria toxin.

Preparation of the Tetanus Toxin

The tetanus bacillus being a strict anaërobe, special means have to be taken to cultivate it, either by replacing the air in the culture flask with hydrogen, or, more convenient still, by cultivating in a sodium sulphindigotate broth. For the former, a simple method devised by the writer may be employed. Glass flasks of various sizes up to a litre have a lateral branch one to two inches in length passing from the middle of their necks, formed by fusing in horizontally a piece of glass tubing, and turning it down at the distal extremity for an inch or so. For the smaller sizes the 'yeast flasks' do excellently.

The neck of the flask is corked with a perforated rubber cork, through which a narrow glass tube passes vertically to the bottom of the flask, projecting two inches above the cork. This projecting portion of the tube and also the end of the lateral tube are plugged with cotton-wool, care being taken that the plugs are loose enough to allow gas to pass freely through them. The flasks are filled with a 1 per cent. grape-sugar bouillon, sterilised, and inoculated after momentarily withdrawing the rubber cork and tube. The air is expelled from the flask and replaced by hydrogen by connecting the vertical glass tube with a Kipp's hydrogen apparatus; the gas bubbles through the bouillon and escapes by the lateral tube.

After the hydrogen has been passing for half an hour, a small capsule or tiny phial containing mercury is applied to the free turned-down end of the lateral tube, and tied on, so that the open extremity of the tube just dips beneath the surface of the mercury. The projecting end of the tube which passes through the rubber cork is then sealed off in the blowpipe flame. The flask is thus filled with hydrogen, and air cannot enter on account of the mercurial valve, but if any gas be formed during the growth of the organism, it can escape. The flask, with the mercurial valve *in situ*, is placed in an incubator at 37° C. After a lapse of two days the bouillon becomes turbid, and gradually a small precipitate, consisting chiefly of bacilli and spores, falls to the bottom ; a surface film never forms. The culture can be used after it has been growing for three weeks, but preferably for a month : after five weeks' growth the virulence seems to diminish.

By the sulphindigotate method, ordinary stoppered bottles filled completely full with the medium may be used, but there is some risk of breakage owing to gas formation, and it is preferable to employ a special bottle such as that devised by Dean. In this form, a glass gutter is moulded on to the neck of the bottle, which can be filled with mercury, and a glass cap fits loosely over the mouth of the bottle and dips into the mercury. In this way a mercury valve is formed, and any gas developing during incubation can escape by lifting up the cap. The medium consists of ordinary bouillon to which 0·3 per cent. of sodium sulphindigotate has been added. The bottle being filled as full as possible, the medium is inoculated and incubated, and the tetanus bacillus will be found to grow well and to produce an excellent toxin. The toxin broth is filtered through a porcelain filter and preserved.

Immunisation of the Horse

Tetanus toxin being extremely active and the horse being very susceptible, extreme caution must be observed during the earlier stages of immunisation. The toxin may be weakened by heating or by mixing with some agent such as iodine; Gram's iodine solution may be employed.[1]

The following illustrates the dosage employed for a pony immunised by the writer.

To commence with, the toxin was weakened by the addition of an equal volume of Gram's iodine solution; of this mixture gradually increasing doses were injected subcutaneously in the neck or shoulder, starting with 0·5 c.c. and going up to 8 or 10 c.c. This took from May 2 to June 22, three injections being given weekly. The next two doses were

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[1] Iodine 1 part, potassium iodide 2 parts, water 300 parts.

4 c.c. and 8 c.c. of a mixture of 2 parts of toxin and 1 part of Gram's iodine solution. After this, commencing July 2, the pure toxin was injected, the dose being 1 c.c., which was increased by degrees until 22 c.c. were injected on July 23. On July 25 4 c.c. were injected into the jugular vein, followed by rather alarming symptoms half an hour after, the animal falling prostrate, with legs extended, laboured respiration, and rapid small pulse. These symptoms, however, lasted only about ten minutes, after which the animal seemed to recover completely. On three other occasions somewhat similar attacks occurred after intravascular injections.

In spite of the apparent risk, the injections into the jugular vein were continued in gradually increasing amounts, and on August 30 the animal received a dose of 70 c.c. without ill-effect. This was in the early days of the preparation of anti-toxins, and I do not think that there is any particular advantage in giving the smaller doses by intravenous injection. When a high immunity has been attained, a large dose of toxin may be given once every week intravenously. Just as in diphtheria, the anti-toxic value of the serum, having attained a maximum, remains at this for a time, then begins to sink, and ultimately becomes almost extinguished. This is the writer's experience, though Salter has stated the contrary.

Standardisation of the Tetanus Anti-toxin

The original method of standardising tetanus anti-toxin was by finding the minimum amount of the anti-toxin which, when injected simultaneously with the minimal lethal dose of tetanus toxin, completely protected a guinea-pig of 500 grams weight. The weight of guinea-pig in grams which is so protected by 1 c.c. of the serum gives the value of the serum in units. Thus if from the tests, 1,000 guinea-pigs, each weighing 500 grams, were protected by 1 c.c., then the value of the serum would be said to be 500,000 (500 × 1,000). But it is better to use *ten* lethal doses of toxin for reasons previously mentioned (p. 80), and a unit was next defined as that amount of serum which would completely protect a 500 gram guinea-pig from ten minimal lethal doses.

Behring has more recently devised a system of testing analogous to that employed for diphtheria anti-toxin.[1] Both the anti-toxin and the toxin for testing can be obtained from the 'Höchst' chemical works. The test toxin

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[1] *Deutsch. med. Woch.*, 1900, No. 2, p. 29.

(described as 'Testgift No. V.') is of such a strength that 1 c.c. would kill after a lapse of four to five days 4,000,000 grams of living mice, 50,000 grams of living guinea-pigs, and 100,000 grams of *young* rabbits. The test anti-toxin (A T) contains 100 units in one gram, the unit being such that

$\frac{1}{1000}$ unit A T + 0·01 c.c. test toxin
dissolved in 0·4 c.c. distilled water
= L_0 dose for the mouse
(L_0 = exact neutralisation, see p. 13).

The exact method of testing is as follows:—

0·1 gram of test anti-toxin is dissolved in 100 c.c. of 0·3 per cent. aqueous carbolic, this is the 'anti-toxin test solution,' and each c.c. contains $\frac{1}{10}$ unit. Into an Erlenmeyer flask 1 c.c. of the anti-toxin solution, 1 c.c. of the test toxin (No. V.), and 38 c.c. of distilled water are introduced, mixed, and allowed to stand for thirty minutes, and 0·4 c.c. is then injected subcutaneously into a mouse weighing about twelve grams (0·1 c.c. for every three grams of body weight); no tetanic symptoms should ensue.

A second mixture is then made, viz.:

1 c.c. of the anti-toxin test solution.
1·1 c.c. of the test toxin (No. V.).
37·9 c.c. of distilled water.

This, mixed and injected into a mouse in the same way, should cause some tetanic symptoms, but not death.

The third mixture:

1 c.c. of the anti-toxin test solution,
1·2 c.c. of the test toxin (No. V.),
37·8 c.c. of distilled water,

should cause the death of the mouse in $3\frac{1}{2}$–5 days.

Behring expresses these results as follows:

1. $\frac{1}{1000}$ anti-toxin unit + 0·01 c.c. test toxin No. V. } L_0 (=neutralisation)
2. $\frac{1}{1000}$ anti-toxin unit + 0·011 c.c. test toxin No. V. } L (= symptoms)
3. $\frac{1}{1000}$ anti-toxin unit + 0·012 c.c. test toxin No. V. } L + (= death)

Having controlled the test toxin with the standard anti-toxin in this way, the anti-toxin to be tested is substituted for the standard anti-toxin and the tests made in the same manner.

This is carried out as follows. One cubic centimetre of the fluid anti-toxin is mixed with 99 c.c. of distilled water in an Erlenmeyer flask. Of this dilution, 1 c.c. is mixed with 38 c.c. of distilled water in another Erlenmeyer flask, and to it is added 1 c.c. of the test toxin No. V. In the case of another toxin, an equivalent amount is added to the 1 c.c. of anti-toxin solution and the whole is made up to 40 c.c. by the addition of distilled water. Of the mixture, 0·4 c.c. is injected into a 12-gram mouse. If the mouse remains unaffected, then the serum contains at least ten anti-toxin units per c.c.; if it becomes tetanic, the serum contains less than ten units per c.c., and further tests have to be made, using stronger dilutions of the serum, until the exact neutralisation point is hit off.

No definite standard seems to have been generally adopted by the various manufacturers; each laboratory appears to have its own standard. No tetanus anti-toxin for therapeutic use should possess a less potency than 1,000,000 as reckoned by the Roux unit, i.e. 1 c.c. should be sufficient to protect 1,000,000 grams of guinea-pig against the minimal lethal dose of tetanus toxin.

Therapeutic Use of Tetanus Anti-toxin

The therapeutic use of tetanus anti-toxin, in spite of its great potency experimentally, has not been attended with such striking results as with diphtheria anti-toxin. Two factors help to explain this somewhat disappointing result. In the first place tetanus toxin is extremely active; in fact, it is by far the most active toxic substance that is known, and minute doses are sufficient to cause irreparable tissue damage. Secondly, tetanus is not recognised until the nerve centres have been attacked by the toxin; spasm of the facial muscles is the first indication of the onset of the disease, and this may be overlooked or be unnoticed until definite tetanic seizures announce that the central nervous system is gravely implicated. As previously stated, anti-toxin cannot repair damage already done, and if this be severe the disease must run its course. In diphtheria, happily, the disease is usually localised and recognisable by the local lesion (membrane &c.) at an early stage, before any great absorption of toxin, with consequent tissue damage, has taken place. There is some difficulty in arriving at a just estimate of the value of antitoxin in tetanus, for there is a considerable difference in the mortality in different cases, some being comparatively mild, and tending to recover spontaneously. Generally speaking, though not always, the severity of the attack is proportional to the duration of the incubation period: the shorter the latter, the more severe the disease. The so-called idiopathic cases[1] are also usually less severe than the manifestly traumatic ones.

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[1] All the forms of tetanus, whether traumatic, idiopathic, or 'rheumatic,' are due to the *B. tetani* of Nicolaier. In the two last named, the bacillus finds a nidus in some minute wound which escapes notice.

The case-mortality from tetanus has been variously estimated. Gowers estimates the mortality in traumatic tetanus at 90 per cent. and in idiopathic tetanus at 50 per cent. Lambert thinks that a fair estimate is 88 per cent. for acute cases, and 40 per cent. for the subacute and chronic ones. He has collected details of a number of cases treated with anti-toxin and gives the following summary: 'We have a total of 279 cases with a mortality of 44·08 per cent., but of these we must rule out 17 cases, made up of 4 deaths from intercurrent diseases, 8 deaths in cases in which the anti-toxin was given but a few hours before death, and 5 recoveries in which anti-toxin was not given until after the twelfth day, as they probably would have recovered without it. We have left 262 cases with 151 recoveries and 111 deaths, a mortality of 42·36 per cent. Dividing the cases into acute and chronic, we have 124 acute cases, with 35 recoveries and 89 deaths, a mortality of 71·77 per cent. and 128 chronic cases with 116 recoveries and 22 deaths, a mortality of 15·94 per cent. In interpreting critically these statistics we see that in acute cases the mortality is but slightly reduced, being but 72 per cent.

instead of 88 per cent. But in the less acute cases there is a decided improvement, from 40 per cent. to 16 per cent.'

The writer some years ago collected details of 50 cases treated with anti-toxin. Only 16 deaths occurred, which gives a mortality of 32 per cent. One of the cases was an idiopathic one, and ended in recovery; another was a case of tetanus neonatorum and ended fatally. The remaining 48 cases were all traumatic, though in two or three instances the incubation period was abnormally long. The details of 5 of these cases were incomplete. Of the remainder (43), 24 had an incubation period of eleven days or under, with 9 deaths; while 19 had an incubation period of more than eleven days, with 3 deaths. Of the acute cases having an incubation period of eleven days or under, this would give a case-mortality of 37·5 per cent.

Leyden states that he has never known recovery to take place when the temperature has risen to 105° F.

Dosage and mode of administration.—In no condition is early treatment of such paramount importance as in tetanus. Immediately there is the slightest suspicion of tetanus, anti-toxin should be given; if tetanus does not ensue, no harm will have been done.

If the case be seen immediately upon the development of the premonitory symptoms, (stiffness &c. of the facial muscles) 20-30 c.c. of the serum may be injected subcutaneously, followed by an injection of 10 c.c. every eight hours as long as the symptoms last. If any time has elapsed since the development of the premonitory symptoms, 10 c.c. should be administered intravenously and 20 c.c. subcutaneously, followed by 10 c.c. subcutaneously every eight hours as before. But if the case has lasted any length of time, and especially if spasms have already occurred, no time should be lost in giving the anti-toxin by intracerebral inoculation.

Tetanus toxin has an especial affinity for the nerve centres, and if injected into the blood stream rapidly (within a few seconds) disappears and becomes ‘anchored’ to the tissues of the central nervous system. By intracerebral injection, therefore, the tetanus anti-toxin is at once enabled to attack or counteract the tetanus toxin *in situ*. This is very important; for, as already stated, anti-toxin is comparatively slowly absorbed from the subcutaneous tissues, and it may be some hours before a subcutaneous dose is completely absorbed. Intravenous injection is more efficacious, but intracerebral inoculation is obviously the one which gives the best hope of success, and in tetanus every moment's delay adds to the risk of failure. Nocard and Roux have shown experimentally that intracerebral inoculation of the anti-toxin is far more potent than subcutaneous in the treatment of tetanus. The anti-toxin may be injected subdurally, into the substance of the cerebral hemispheres, or preferably into the lateral ventricles. Barker describes a successful case of the former mode of injection; the skull was trephined with a $\frac{1}{4}$-inch trephine and 7·5 c.c. of the anti-toxin injected, while on each of the succeeding four days 20 c.c. was injected subcutaneously in the flanks.

Semple records a case treated by the second method with recovery. An imaginary line is taken over the scalp from one auditory meatus to the other, a second line is taken from the base of the nose to cross the first at right angles on the top of the head, and a third one from the outer angle of the orbit to the point of intersection of the first two lines. The centre of the last line is the seat of operation. A small incision is made through the soft tissues down to the bone and the edges of the wound are held open. Then a hole slightly larger than the needle of the syringe is drilled through the bone by means of an archimedean drill having a moveable collar so that the depth to which the drill should penetrate may be regulated. The needle should be about two inches long and should have a blunt rounded point to avoid the risk of wounding a blood-vessel, and may be attached to the syringe by a short length of rubber tubing. If such a needle be not available, the ordinary syringe needle may be converted into one by filing it down after heating it to redness in a spirit lamp or gas flame, and slowly cooling in order to soften the steel. The needle is then passed into the brain perpendicular to its surface and 2·5 c.c. of the anti-toxin injected *very slowly*. The injection should occupy at least ten minutes, in order that absorption may take place and the brain tissue be undisturbed. Another similar dose should then be injected in the same manner on the other side, 5 c.c. of the anti-toxin being thus given. It is preferable to use a *concentrated* anti-toxin, so that as much as possible may be administered. For this purpose the *dried* preparation may be used and should be dissolved in *half* the usual quantity of sterile water, viz. 1 gram in 5 c.c. Only a single dose is given intracerebrally; a subcutaneous dose of 20 c.c. is given at the same time, and this is followed by 10 c.c. thrice daily as long as severe symptoms are present.

For injection into the lateral ventricle the following method may be adopted. A point is taken 1¼ inch behind and 1¼ inch above the centre of the auditory meatus. Here the bone is drilled as for intracerebral injection, and the needle is passed into the brain for 5–6 centimetres (2–2½ inches) in a direction pointing to the tip of the auricle of the opposite side. The anti-toxin is then injected as for intracerebral inoculation. Letour records five cases of tetanus in which recovery followed intracerebral administration of the anti-toxin. The serum was used freely, as the following details of the dosage show :

	Case I.	II.	III.	IV.	V.	
Subcutaneous	110	60	130	—	—	c.c.
Intracerebral	20	34	48	13	19	c.c.

(quoted by Grünbaum, *Practitioner*, Nov. 1902, p. 621). Bates also records a case of recovery following the administration of 5 c.c. into the lateral ventricles and 35 c.c. subcutaneously (*Lancet*, 1902, i. p. 227).

As a modification of intracerebral inoculation, intraspinous inoculation may be mentioned, the anti-toxin being injected into the subarachnoid space of the spinal cord. This may be done by means of lumbar puncture ; 10 c.c. of the cerebro-spinal fluid is drawn off, and a similar amount of anti-toxin injected. Leyden has reported a case successfully treated by this method. D'Ancona has also treated two severe cases by this method ; four injections were given in each case, in the first on the 13th, 15th, 19th, and 22nd days, and in the second on the 5th, 8th, 9th, and 12th days.

In all cases of tetanus, in addition to anti-toxin, general treatment should be adopted. The wound, if there be one, should be excised, if the situation permit, or opened up and scraped and cleansed, and it might be useful to thoroughly swab it out with a solution of iodine (*e.g.* Gram's solution, p. 113), which destroys the tetanus toxin. It is doubtful whether so severe a procedure as the amputation of the limb in which the wound is situated is of any use, though from recent experiments by Marie and Morax the tetanus toxin seems to spread along the nerve trunks.

A full dose of chloral and potassium bromide (gr. 30 of the former and gr. 40 of the latter) should be given at once and repeated as occasion requires. Antimony and morphine may also be given, gr. $\frac{1}{8}$–$\frac{1}{6}$ of each every two hours. Chloroform anæsthesia should be used if deglutition induces spasm. Amyl nitrite is valuable for reducing the dangerous spasm of the glottis and respiratory muscles ; capsules should be kept by the bedside and a drop or two applied to the nasal mucous membrane. The patient should be kept in a quiet darkened room and should have abundant fluid nutriment ; plenty of fluid is indicated in order to promote the elimination of the tetanus toxin.

Prophylactic Use of Tetanus Anti-toxin

Many lives might be saved if the prophylactic use of tetanus anti-toxin were more general. In any wound likely to be followed by tetanus, *e.g.* lacerated and contused wounds soiled with earth, and especially if neglected, foul and suppurating, and in 'tetanus districts,'[1] injections of tetanus anti-toxin may be given with the certainty of preventing the onset of tetanus.

Since anti-toxin does not immunise for more than three weeks, and since the incubation period of tetanus may be as long as one month, at least two injections of the anti-toxin should be given, 10 c.c. immediately and 10 c.c. a fortnight afterwards, and to be on the safe side a third injection may be given a fortnight after the second one.

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[1] In certain districts, as in the West Indies and West Coast of Africa, tetanus is extremely frequent.

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Anti-Venene (Anti-venomous Serum)

The chief venomous snakes are the common viper found throughout Europe, the asp and cerastes in Africa, the cobra, hamadryad, krait, bungarus, and Russell's viper in India, the rattlesnake, copperhead, water mocassin, coral snake, and lance snake of America, and the tiger-snake, black snake, and death adder in Australia. The active constituents of snake venom are proteid in nature, consisting of a globulin-like body and of a peptone-like body, the relative proportions of which differ very much in different venoms. Cobra venom, for example, contains 2 per cent. of the globulin and 98 per cent. of the peptone, whereas rattlesnake venom contains 25 per cent. of the globulin and 75 per cent. of the peptone. The two substances differ in their action, the globulin producing especially the local symptoms, swelling, pain, injection, hæmorrhages, and hæmolysis, while the peptone gives rise to the general symptoms, twitching, convulsions, and paralysis, particularly of the respiratory centres. The potency of the various venoms—that is, the smallest amount which can be relied upon to cause the death of the rabbit when injected subcutaneously—is as follows :

Tiger-snake	venom	0·00005	gram	per kilo	of rabbit.
Death-adder	,,	0·0002	,,	,,	,,
Cobra	,,	0·00025	,,	,,	,,
Rattlesnake	,,	0·004	,,	,,	,,
Viper	,,	0·004	,,	,,	,,

It is to be noted that the susceptibility of different animals varies very considerably ; thus, Fraser found that the minimal lethal dose of cobra venom per kilogram of body weight was for the guinea-pig 0·00018 gram, for the rabbit 0·000245 gram, for the white rat 0·00025 gram, for the cat somewhat less than 0·005 gram, but for a six-weeks-old kitten 0·002 gram, and for the grass snake 0·03 gram. The mongoose is perhaps relatively slightly less susceptible than are other animals, but it owes its immunity when attacking the cobra to its agility, whereby it escapes being bitten. The amount and activity of a snake's venom vary somewhat at different seasons and under different conditions, and after biting a few times in rapid succession the venom becomes exhausted.

For the preparation of the anti-venene the horse is the animal chosen, and is immunised by the cautious injection of repeated doses of venom. The venom is for the most part collected from snakes that have been killed, being expressed from the poison gland through the fangs and collected on a dial glass. Or the venom glands may be dissected out, the venom dissolved from them by means of distilled water, and the solution evaporated to dryness *in vacuo* over sulphuric acid.

From living snakes kept in captivity the venom can be obtained by seizing the snake by the head with a long pair of tongs with flattened extremities covered with india-rubber and thus lifting it from the cage without risk. It is then grasped with the left hand immediately behind the head, so that it cannot bite, and a shallow dish or dial glass, preferably covered with rubber, being presented to it, the snake strikes, and the venom is caught on the vessel; this procedure may be repeated two or three times, and a further quantity may be expressed from the glands by applying pressure (‘milking’). The venom should then be dried in a desiccator over strong sulphuric acid, and in the dry state may be kept almost indefinitely without diminution in toxicity. For use a weighed quantity is dissolved in sterile distilled water.

The immunisation of the horse is an extremely tedious process: in the earlier stages because the dose must be increased very slowly and cautiously; in the later ones, when a large dose has been attained, on account of the difficulty of procuring a sufficient supply of the venom. In the earlier stages, the process of immunisation may be accelerated by giving some preliminary injections of anti-venene.

The dosage of venom for immunising may be illustrated by the following example given by Tidswell for that of the Australian tiger snake:

The treatment was commenced on June 7, 1898, by the subcutaneous injection of ·0005 gram of the venom. This was repeated in a week, and a week later the dose was increased to ·00075 gram. Increments of ·00025 at each dose were maintained during the first six months of the treatment, but after that they were greater, viz. ·0005 (January 1899), ·01 (March 1899), ·05 (May 1899), and ·1 (January 1901), gram. The increments were pretty regularly given, the same dose being repeated only on the rare occasions when the reaction was more than usually pronounced. Between October 1899 and May 1900 the pressure of other work interfered with regular treatment, but otherwise the horse was injected once a week (June 1898 to April 1899); once a fortnight (May 1899 to May 1900); and once a month (July 1900 to January 1902). The lengthening of the intervals was due to the difficulty of collecting the larger amounts of venom required as the dose increased. This same difficulty limited the maximum dosage to ·6 gram, which was reached in April 1901, and which it was not possible to maintain more than approximately. During the period of three and a half years covered by the treatment the horse received a total quantity of about 10 grams of pure tiger-snake venom. The dose which the horse could finally bear without effect (0·6 gram) was about equal to the aggregate yield of twenty-one or twenty-two average snakes, and the total amount received by the horse during the treatment was about equal to the amount which would be yielded by 333 average snakes.

Calmette commences immunisation with 1 milligram of cobra venom.

Standardising the Anti-venene

Calmette points out that neither the method of Behring nor that of Roux employed for testing diphtheria and tetanus anti-toxins (see p. 80) is applicable in the case of anti-venene, because (1) the sensibility of various animals to snake venom is very different, (2) the toxicity of the venom is different for each species of venomous serpent, and for the same species varies with the time of collection, and (3) the amount of anti-venene required to immunise different animals varies inversely as their resistance. Thus about twelve times the amount of serum is required to immunise a guinea-pig of 500 grams weight against the minimal lethal dose of venom than is required to immunise a rabbit weighing 2,000 grams. The fowl is one of the most sensitive of animals to the venom. Calmette therefore suggested and adopts the following method of standardisation :

1. The amount of dry venom which is certainly lethal in fifteen to twenty minutes for the rabbit is determined, the dose being dissolved in sterile water and injected into the marginal vein of the ear. This varies from 0·5 milligram (*Bungarus cœruleus*) to 6 milligrams (viper).

2. A series of rabbits each of about 2 kilograms in weight is injected intravenously (by the aural vein) with increasing amounts of the anti-venene, 0·5 c.c., 1 c.c., 2 c.c., 3 c.c., &c.

3. A quarter of an hour after the injection of the serum, the lethal dose of venom is injected into the other aural vein. If 1 c.c. of the serum suffices to keep alive a rabbit weighing 2,000 grams, the serum is said to contain 2,000 units per c.c. or 20,000 units in 10 c.c.

Semple and Lamb raise the following objections to Calmette's method: (1) that there is no direct estimation of the amount of venom which a given quantity of serum will neutralise; (2) that no estimation is made of the amount of venom the injection of which an untreated animal is capable of surviving; and (3) that Calmette's test dose for the rabbit amounts to about three lethal doses for that animal.

Calmette apparently uses both for immunising and for standardising a mixture of venoms heated to 73° C. for half an hour and then filtered. Myers has pointed out that it would be preferable, in order to obtain a correct estimate of the curative value of the serum, to use an unheated venom, and he found that mice would do quite as well as rabbits for testing purposes. He also showed that more accurate results are obtained by mixing the venom and serum *in vitro* than by Calmette's method. Lamb and Hanna employ rats weighing about 115-120 grams for testing purposes. They are larger and more easily managed than mice and are very susceptible, the minimal lethal dose of cobra venom being at the rate of 0·33 milligram per kilogram of body weight. The venom for testing is obtained by 'milking' the snakes and drying in a desiccator. For use it is powdered, again dried over sulphuric acid, and weighed out, a 0·1 per cent. solution being made in physiological salt solution. The certain minimal lethal dose for rats of the above weight was found to be 0·04 milligram and 0·035 milligram was the maximum non-lethal dose, and for testing ten times the certain lethal dose was used, viz. 0·4 milligram. This test-dose of venom was mixed *in vitro* with varying amounts of the serum to be tested, allowed to stand for not less than half an hour at the laboratory temperature (25° C.), and then injected subcutaneously.

The following table (table V., p. 136) given by Lamb and Hanna illustrates the method and the results obtained.

From this testing it is seen that 0·5 c.c. of this serum failed to neutralise 0·4 − 0·035 = 0·365 milligram, while 0·6 c.c. neutralised at least this amount; therefore 1 c.c. would neutralise 0·61 milligram of cobra venom.

TABLE V.—EXPERIMENTS TO ASCERTAIN THE AMOUNT OF VENOM WHICH ONE CUBIC CENTIMETRE OF COMPARATIVELY FRESH ANTI-VENOMOUS SERUM WAS CAPABLE OF NEUTRALISING

Animal	Amount of dried venom in milligrams	Amount of serum in cubic centimetres	Result
Rat 1	0·4	0·2	Died in 2 hours
2	0·4	0·3	,, ,, ,,
3	0·4	0·4	Died within 42 hours
4	0·4	0·5	,, ,, ,,
5	0·4	0·5	Died within 46 hours
6	0·4	0·6	Ill for one day; recovered
7	0·4	0·6	No symptoms
8	0·4	0·7	,, ,,
9	0·4	0·7	,, ,,
10	0·4	0·8	,, ,,

Specificity of Anti-venene

The specificity or otherwise of anti-venene is of considerable importance. Calmette for a long time maintained that anti-venene is not specific; that is, cobra anti-venomous serum, though acting most potently against cobra venom, would also antagonise other venoms. Some experiments by Martin threw considerable doubt upon this, and the further ones of Tidswell conclusively show that Calmette's anti-venene, which is prepared mainly with cobra venom, though small quantities of other venoms are also used, has little or no neutralising power against tiger-snake venom, and that his own tiger-snake anti-venene does not neutralise the venoms of other Australian snakes, viz. those of the black snake, brown snake, and death adder.

Therapeutic Use of Anti-venene

The action of snake venom is so rapid that unless the case be seen soon after the bite, the chances of successful treatment are remote. A ligature should at once be applied above the bite, if the locality permit. The wound, if necessary, should be opened up and thoroughly washed out with a solution of chromic acid (1 per cent.), or, still better, a solution of chloride of gold (1 per cent.), or a freshly prepared solution of chloride of lime (1 in 60). The two last-named substances are most efficacious in destroying any venom that may not have been absorbed.

Without delay an injection of anti-venene should be given subcutaneously, or, if symptoms of intoxication have already supervened, intravenously into a superficial vein. Calmette recommends that from 10 c.c. to 20 c.c. should be given. But Lamb and Hanna calculate from their experiments that about 37 c.c. of Calmette's serum would be required to neutralise the full dose of venom which a cobra could inject at a bite, assuming man to be as susceptible as the most susceptible animal.

Fraser and Calmette, however, consider that the lethal dose for man approximates probably to that for the cat or dog, rather than to that for the vegetable feeders, the rabbit and guinea-pig, and this might account for the many cases of snake-bite reported to have been cured with comparatively small amounts of anti-venene. But to be on the safe side, at least 30 or 40 c.c. of the anti-venene should be injected in every case of cobra-bite, and a still larger dose if symptoms have already ensued. Symptomatic treatment should also be employed, stimulants, such as alcohol (in moderate amount, so as to avoid any narcotic effect), ether, ammonia, strong coffee, with strychnine hypodermically, and electricity if respiratory paralysis threatens.

A number of cases of snake-bite successfully treated by anti-venene have been reported ; references to some of these are given in the bibliography below.

The experiments of Martin and of Tidswell mentioned above show that the anti-venene prepared by Calmette at Lille, which is practically the only make on the market, can hardly be relied upon as an antidote to any other venom than that of the cobra.

N.B.—The anti-venene must be as fresh as possible, for Lamb and Hanna have found that it undergoes a progressive and fairly rapid deterioration when stored in hot climates, and that this deterioration is greater and more rapid the higher the mean temperature to which it is subjected.

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CHAPTER V.

THE ANTI-MICROBIC SERA—ANTI-STREPTOCOCCIC SERUM — ANTI-PNEUMOCOCCIC SERUM — ANTI-PLAGUE SERUM—INJECTION OF ANTI-STREPTOCOCCIC AND ANTI-PLAGUE SERA—ANTI-TYPHOID SERA—ANTI-TYPHOID EXTRACT OF JEZ—OTHER SERA

In the previous chapter the chief anti-toxic sera have been dealt with. We now have to consider those diseases the specific micro-organisms of which produce little or no toxin, and the sera for which are mainly anti-microbic in nature. The chief of these are streptococcic infections, pneumonia, plague, and enteric or typhoid fever.

Anti-streptococcic Serum

Marmorek was the first to prepare an anti-serum for streptococcic infection. The *streptococcus pyogenes* or *erysipelatis* (the two forms are generally regarded as being identical) is the organism met with in erysipelas and in certain forms of pyæmia and spreading septic infections.

It forms but little toxin in cultivations, and immunisation has to be carried out by means of the cultures.

Preparation of the Streptococcus Cultures

Enhancing the virulence.—In the first place, the virulence of the streptococcus has to be raised by a succession of passages through a susceptible animal, the rabbit being that generally employed. The mouse may also be used for the earlier inoculations, and when a fair degree of virulence has been attained the rabbit is substituted. Of an ordinary streptococcus just isolated 0·5 c.c. of a thirty-hours broth culture, injected subcutaneously, may be required to kill a mouse, or 1 c.c. intravenously a rabbit. When the animal dies, the heart-blood, obtained in a glass pipette, is injected into a second animal, and so on. The inoculations may be carried on from animal to animal, without cultivation on artificial media, or the heart-blood may be inoculated into the special serum broth (see below), and cultivated for forty-eight hours, and this culture then inoculated into the second animal, and this process repeated again and again. Aronson found that, by inoculating rabbits with broth cultures of feebly virulent streptococci together with a small amount of diphtheria toxin, the animals died, and the subcultures from them were then found to have attained sufficient virulence to kill without the aid of the toxin, and the organisms were further increased in virulence by a succession of passages. Bulloch recommends cultivating for eighteen hours, inoculating after the first two or three passages subcutaneously in the abdomen, and subculturing from the liver. The number of passages required varies from about twenty to thirty, at the end of which time the virulence does not become exalted by further passages; the culture may therefore be termed a 'fixed virus.' The virulence has now reached an extraordinary pitch; according to Marmorek, one thousand-millionth ($\frac{1}{1000000000}$) of a cubic centimetre of broth culture will kill a large proportion of the rabbits inoculated with it, while one hundred-millionth ($\frac{1}{100000000}$) of a cubic centimetre is invariably fatal. If the dose be larger, *i.e.* 0·1 c.c., the animal dies within a few hours. The culture needs to be continuously passed through rabbits in order to keep up the virulence. Bokenham states that he is by no means sure that extreme virulence is necessary, and considers that the conditions of cultivation and of the culture-medium may perhaps be of greater importance.

Preparation of the Cultures.—Ordinary broth is not a suitable culture-medium, as in it the virulence of the streptococcus is rapidly lost. The best medium of all consists of human blood-serum 2 parts, and ordinary peptone beef broth 1 part. Since human blood-serum is difficult to obtain, ascitic or pleuritic fluid may be substituted, 1 part of this and 2 parts of beef broth. Failing this, ass's or horse's blood-serum may be used, preferably the former, 2 parts, beef broth 1 part. Veal broth may be substituted for beef broth with advantage. As a culture-medium, Aronson uses a bouillon made from horse-flesh and containing 0·5 per cent. sodium chloride, 0·5 per cent. peptone, and 0·1 per cent. glucose, the reaction being neutral to phenol-phthalein, but alkaline to litmus. The cultures are grown for from two to three weeks, and then the whole, *i.e.* unfiltered, cultures, containing both microbes and toxin, are used for the inoculations. Bordet grows the cultures for twenty-four hours only. For the earlier injections the cultures are killed by heating to 58°–60° C. in a water-bath for one to three hours, care being taken that the cultures are thoroughly mixed during the sterilisation, so as to ensure all parts being raised to the lethal temperature. Subsequently, when some immunity has been attained, the living cultures may be employed, and are injected intravenously to avoid the formation of abscesses, commencing with small doses.

Immunisation

The horse, mule, or ass may be employed; the horse, however, is much less susceptible than the ass to the toxic action of the cultures. With the horse, the treatment may be commenced by an injection of 3–5 c.c. of the killed culture. Since horses vary very much in sensitiveness, it may be well first to give a single cubic centimetre in order to gain some idea of the reaction that will occur. This induces a rise of temperature of 2–4 degrees. A second injection is given when all reaction produced by the first one has disappeared. The dose is gradually increased, the aim being to produce some amount of reaction with each injection.

The process of immunisation is a tedious one and six to twelve months' treatment is necessary before a sufficiently active serum is obtained. Ultimately a dose of 100-200 c.c. of culture is administered.

The period of bleeding the horse is important, since Marmorek found the serum to be toxic up to fifteen days after the last inoculation;

Bulloch, however, did not find it toxic for a longer period than twenty-four hours after the inoculation. Perhaps the period may differ with different strains of the organism, and it would be well to test this point in all cases by inoculating rabbits subcutaneously with 5 c.c. of the serum, taken at varying intervals after the last inoculation.

Polyvalent Serum

Marmorek and others have affirmed the unity of streptococci obtained from various sources, whereas Piorkowski has found that one streptococcus may not protect against another. This is probably the case, and it would be well to conduct the immunisation with several strains of the streptococcus isolated from different sources, as has been done by Aronson and by Tavel.

Standardising the Serum

The method of standardisation is to inject a measured volume of the serum subcutaneously, and, at the same time, 10 minimal lethal doses of the streptococcus intravenously, into a rabbit. Not more than 0·05 c.c. of serum should be required to preserve the life of a rabbit.

Therapeutic Use of Anti-streptococcic Serum

The therapeutic use of streptococcic serum in septic and puerperal infections, it must be confessed, has not been followed by that benefit which was at one time anticipated. This is due partly to the fact that these conditions are not solely due to streptococci, but may be dependent upon infection with a number of other organisms, *e.g.* various staphylococci, the *micrococcus tetragenus*, pneumococcus, *bacillus coli*, gonococcus, &c. An anti-serum being specific, anti-streptococcic serum will act only in a streptococcic infection, and, as mentioned above, an anti-serum for one strain of streptococcus may not be active towards another strain. Lastly, it has to be remembered that an anti-microbic serum, such as anti-streptococcic serum, is never as potent as an anti-toxic serum. Nevertheless, in any septic infection the use of anti-streptococcic serum should be tried; it will do no harm, and three or four doses will prove whether it will do good. A dose of 10 to 20 c.c. should be given twice daily, and if any decided effect upon the temperature and general condition ensues, it should be persevered with (see also p. 171). Some continental authorities have suggested giving far larger doses (up to 150 c.c.) than those here suggested. *The serum should be obtained as fresh as possible; it probably rapidly deteriorates with keeping.*

It is in cutaneous erysipelas perhaps that the best results are obtained with anti-streptococcic serum: a dose of 10 c.c. given twice daily usually cuts short the attack within two or three days. The writer has obtained excellent results with the serum treatment in this disease. As regards puerperal infection, the following conclusions were formulated by a Committee of the American Gynæcological Society in 1899 on the value of anti-streptococcic serum:—

'1. A study of the literature shows that 352 cases of puerperal infection have been treated by many observers, with a mortality of 20·74 per cent.; where streptococci were positively demonstrated, the mortality was 33 per cent.

'2. Marmorek's claim that his anti-streptococcic serum will cure streptococcic puerperal infection does not appear to be substantiated by the results thus far reported.

'3. The personal experiences of your committee have shown that the mortality of streptococcic endometritis, if not interfered with, is something less than 5 per cent., and that such cases tend to recover if Nature's work is not undone by too energetic local treatment.

'4. The experience of one of the members of the committee with anti-streptococcic serum has shown that it has no deleterious effect upon the patient. But we find nothing in the clinical or experimental literature or in our own experience to indicate that its employment will materially improve the general results in the treatment of streptococcic puerperal infection.'

As regards the serum treatment, it is remarked that, if any benefit is to be derived from the use of anti-streptococcic serum in a given case of infection, it will respond to the injection of 20–30 c.c., and from 30–50 c.c. will control responsive cases if treatment be commenced early.

Prophylactic Use of Anti-streptococcic Serum

The prophylactic use of anti-streptococcic serum has been suggested by Watson Cheyne and others before operation upon the mouth and throat to prevent the septic pneumonia which is so frequent a concomitant in these procedures; also in operations upon the rectum. A dose of 10 c.c. should be given on each of the three days preceding the day of operation.

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Anti-pneumococcic Serum

G. and F. Klemperer first immunised rabbits against the pneumococcus by the intravenous injection of a broth culture, the vitality of which had been destroyed by heating to 60° C. for one to two hours, and subsequently inoculating the animals with living cultures. During this treatment many animals may succumb, but those which recover are rendered immune to subsequent inoculation. This is probably the best method, but others may be used ; for example : injection with filtered cultures, or of a glycerin extract of blood from a case of pneumococcic infection, or by inoculation with attenuated cultures. Washbourn was the first in this country to immunise a horse against the pneumococcus. The pony was injected with broth cultivations that had been heated to 60° C. for one hour, commencing with about 50 c.c. injected subcutaneously in the shoulder, the injections being repeated as soon as all reaction caused by the preceding injection had passed off. Afterwards living broth cultivations and emulsions of living agar cultures were employed. The following data given by Washbourn indicate the method of procedure and the effect upon the animal:

November 21, 1895: 70 c.c. broth cultivation heated to 60° C. for one hour. The temperature rose next morning to 103·5° F., but soon subsided.

November 30: 170 c.c. broth cultivation heated to 60° C. for one hour. No pyrexia and no local reaction.

December 7: a small loopful of a living agar cultivation. No local reaction.

December 13: the whole of a living agar cultivation. No local reaction.

December 19: two living agar cultivations. No local reaction.

January 4, 1896: six living agar cultivations. A swelling appeared at the seat of injection and lasted five days.

January 13: 50 c.c. living broth cultivation.

A swelling of the size of an orange appeared at the seat of injection and lasted a few days.

January 21: 82 c.c. living broth cultivation. A large swelling appeared at the seat of inoculation and lasted nine days.

February 7: 150 c.c. living broth cultivation. A diffuse swelling appeared, but soon subsided. The temperature reached 104·2° F.

February 21: 100 c.c. living broth cultivation injected into both sides of the neck. A swelling appeared on both sides of the neck, more marked on the right side. This subsided in a few days. The temperature reached 103° F.

March 3: 112 c.c. living broth cultivation injected into both sides of the neck. The animal appeared ill for twenty-four hours, and there was some swelling at the seat of injection.

March 14: 150 c.c. broth cultivation injected into both sides of the neck. Some swelling occurred on both sides, but soon disappeared. Animal ill for twenty-four hours.

March 27: 200 c.c. broth cultivation injected into both sides of the neck. Marked swelling occurred on both sides, and lasted two days.

The animal then developed an abscess in the near foreleg, due to streptococcic infection, and the injections were discontinued, but five months afterwards it was found that 0·03 c.c. of the serum protected against 10 lethal doses of the pneumococcus.

Pane has in a similar manner prepared a highly active anti-pneumococcic serum which has been found by Washbourn and Eyre to possess the protective and immunising powers claimed for it.

Different strains of the pneumococcus are found to vary considerably in virulence, and it is necessary to employ a virulent one, which may be obtained by passage through a succession of rabbits (8–12). The virulence is easily lost on artificial cultivation and must be kept up by an occasional passage through two or three rabbits, and the culture-medium for subculturing should always be agar smeared with blood, preferably rabbit's blood.

Further, distinct varieties of the pneumococcus exist—thus Foa found that animals immunised against one variety of pneumococcus were not necessarily immunised against another, and Washbourn and Eyre that, whereas Pane's serum protected against four out of five varieties of the pneumococcus examined, it possessed no protective power against the fifth, which was a typical pneumococcus obtained from a fatal case of pneumonia. The serum should therefore be a ‘polyvalent’ one.

The horse having been treated for a long period of time, five to nine months, the strength of the serum must be estimated. This is done by obtaining a pneumococcus of fixed virulence—that is, one the virulence of which after a succession of passages through rabbits is not increased by further passages. The pneumococcus of fixed virulence having thus been obtained, a special culture-medium is employed, so that the virulence may be preserved unaltered. This is a nutrient agar having a definite reaction smeared with sterile rabbit's blood, and the cultures are carefully capped and preserved in the incubator at a temperature of 37·5° C. The nutrient agar during its preparation is never heated above 100° C., and after it is made it is neutralised with caustic soda solution, rosolic acid being used as the indicator. After neutralisation 4 c.c. of normal caustic soda solution are added to each litre of the agar. Cultivations twenty-four hours old are used, and platinum loopfuls of the growth are made into an emulsion with a known quantity of sterile broth, so that by dilution any fraction of the contents of the loop can be obtained. The same-sized loop is used throughout, and should contain 0·5 milligram of the growth. First of all the minimal fatal dose of the culture is ascertained by injecting varying fractions of a loopful into the peritoneal cavity of rabbits. Having done this, measured quantities of the serum to be tested are mixed with *ten times* the minimal fatal dose of the culture, and the mixtures immediately after being made are injected into the peritoneal cavities of rabbits. The smallest quantity of serum which protects the animal under these circumstances Washbourn terms a unit. For instance, if 0·03 c.c. of the serum mixed with ten times the minimal fatal dose protects, then each cubic centimetre of the serum contains approximately 33 units. Usually 0·000001 of a loop represented the minimal fatal dose, therefore 0·00001 loop was the quantity mixed with the serum. Occasionally the minimal fatal dose was the tenth of that stated, viz. 0·0000001 loop, as was the case in the following testing given by Washbourn :

		grms.		
Rabbit	O	3,000	0·0000001 loop (control) . .	Died in 36 hrs.
,,	P	3,600	0·000001 ,, ,, . .	,, 60 ,,
,,	Q	3,000	0·000001 ,, +0·03 c.c. serum	Not affected
,,	R	1,600	0·000001 ,, +0·015 ,,	Died in 36 hrs.

Minimal fatal dose = 0·0000001 loop.

With this same serum it was found that 66 units protected against ten minimal lethal doses of the pneumococcus when injected five to six hours, but only delayed the fatal event when injected eight or twelve hours, after the infection.

Therapeutic Use of Anti-pneumococcic Serum

Anti-pneumococcic serum must necessarily have a limited therapeutic use, though if it can be obtained it might be employed in elderly, debilitated, or otherwise unhealthy subjects in whom the disease is likely to run a serious course. Pane's serum seems to be the most potent. Probably a dose of 20 to 30 c.c. should be given subcutaneously twice daily until convalescence is established.

Wilson describes the treatment of 18 cases of acute croupous pneumonia with the anti-pneumococcic serum. The serum was not used to the exclusion of other treatment, which consisted in the systematic administration of Dover's powder, ice-bags to the affected region, the use of calomel, strychnine, alcohol, and inhalation of oxygen when necessary, and other symptomatic treatment as seemed judicious. Of the 18 cases four died—a mortality of 22·2 per cent. In two of the fatal cases no improvement followed the administration of the serum; in the other two slight improvement followed the earlier doses, but the later injections were without effect. The defervescence was by crisis or rapid lysis. The duration of the attack varied between five and fourteen days, the majority of the cases coming to an end on the sixth, seventh, or eighth day.

The summary of the dosage is interesting. In the earlier cases the serum was administered somewhat timidly, and at considerable intervals, the effect being closely watched; later it was given freely and repeatedly. In the first four cases the age of the serum—that is, the period that had elapsed from the time the serum was drawn from the animal until its employment therapeutically—was not ascertained. In the subsequent cases this was known and recorded. The serum in all the cases was administered hypodermically, and the total quantity given varied from 20 c.c. to 460 c.c., 3 cases each receiving 400 c.c. or more. The administration extended over a period varying from six hours to eight days. The age of the serum, as above stated, varied from seven to fifty-three days. The immediate effects were more favourable and more marked in recently drawn serum than in that which had been drawn for longer periods. They consisted, in general, in lowering of the temperature and pulse frequency, mitigation of pain, and tendency to drowsiness. Several of the patients expressed themselves as feeling better after the injection, and seemed to be anxious for the time when it should be repeated. No ill effects were observed that could be ascribed to the trikresol present in the serum. The average duration of the stay in hospital of 13 out of the 14 cases that recovered was twenty and a half days. Of 20 patients admitted into another hospital during the same period, and treated in the ordinary manner without serum, 4 died—a mortality of 20 per cent.—so that it cannot be said that the mortality was lessened by the use of the serum.

Six cases of pneumonia treated with anti-pneumococcic serum with one death are described by Tyler. He found toxæmic symptoms to be completely absent in cases treated with serum; but it was doubtful if the serum had any effect upon the condition of the affected lung, though it seemed to prevent the involvement of fresh areas. He gave 20 c.c. of the serum every six to eight hours until the temperature reached the normal.

Tyler has also collected the records of 141 cases of pneumonia treated with anti-pneumococcic serum, with 121 recoveries and 20 deaths

—a mortality of 14·18 per cent. Excluding several alcoholic, cardiac, and other cases, 127 are left with 6 deaths—a mortality of only 4·7 per cent.

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Diphtheria Anti-toxin in Pneumonia

Talamon has treated 50 cases of acute pneumonia with injections of diphtheria anti-toxin with only 7 deaths, or a mortality of 14 per cent. Under similar conditions, but with ordinary treatment, the mortality during the preceding year had been 37 per cent. The cases were unselected, of all ages and of all degrees of severity, including 8 alcoholic ones. Twenty-five of the patients came under treatment from the second to the fifth day, and among these the mortality was only 4 per cent. Usually two or three injections of 20 c.c. each are necessary for patients under 50 years of age and four or five for those above this age. The course of the temperature is the guide as to the frequency of the injections. Usually each injection is followed the next morning by a lowering of the temperature; should the temperature continue to fall towards evening, an injection is not necessary; should it tend to rise, then the injection should be repeated. The total amount of the serum used in a patient coming under treatment early should not exceed 20, 40, or 60 c.c., according to the age. In grave cases there should be no hesitation in injecting 20 c.c. morning and evening, to be repeated the following day should the temperature not fall.[1]

Legros has not been able to observe any beneficial result from the use of diphtheria anti-toxin in mice infected with cultures of the pneumococcus.[2] It is to be noted that experimental infection is probably very different from the pulmonary infection or pneumonia in man.

O'Malley and Paton have also used diphtheria anti-toxin in various infective conditions, and in broncho-pneumonia of children.[3] It may be of some use, and if so probably acts by stimulating phagocytosis and improving nutrition.

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[1] *La Sem. Méd.*, February 27, 1901, p. 69.

[2] *Ibid.* May 8, 1901, p. 158.

[3] See ref. in *Treatment*, March 1903, p. 36.

Anti-plague Serum

An anti-plague serum was first prepared by Yersin in 1895, since when several modifications have been introduced by different workers.

Preparation of the Anti-plague Serum

In Yersin's method increasing quantities of living agar cultures were injected intravenously into a horse. The method now employed at the Pasteur Institute is as follows: A virulent plague bacillus is made use of, and it is well to employ many strains of the bacillus isolated from different epidemics. The organism must not be passed through animals, or its virulence, though enhanced for one species, may be diminished for another. The virulence may be maintained unimpaired by cultivating on surface agar; a tube is inoculated and incubated at 37° C. for twenty-four hours, and is then kept at room temperature, and a fresh subculture is made every week.

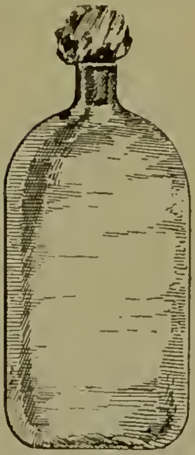

FIG. 19.
'PLATE' BOTTLE.

The material for inoculating the horses is obtained by growing the bacillus on the surface of agar in flat bottle-shaped flasks (fig. 19), the broad side of which has an area of 20 × 10 centimetres and is coated with nutrient agar. An ordinary surface agar tube culture of 18–24 hours' growth is used for inoculating. A little sterile broth or physiological salt solution is introduced into the tube, and an emulsion of the growth prepared, and 1 c.c. of this emulsion is sprayed over the surface of the agar in one of the flat flasks by means of a sterile glass pipette, and the flask, laid flat, is incubated at 37° C. for from thirty-six hours to three days. A good growth having been obtained, 20 c.c. of physiological salt solution are introduced into the flask, and an emulsion of the growth made. The emulsion is then filtered through a layer of sterile cotton-wool to remove particles.

In the earlier stages of the inoculation this emulsion is heated to 65° C. for one hour, in order to kill the bacilli, care being taken that the whole of the fluid attains and is kept at this temperature for this time. The first dose is a little less than 1 c.c. of this killed emulsion, and about a week is usually allowed to elapse between the injections. The dose is gradually increased until, at the end of about three months, the bactericidal power of the blood will have increased to such a degree that if living bacilli be injected they will be almost immediately destroyed. Then the living cultures are injected, one dose every week, until at the end of about six months the whole growth from one flask may be injected without causing symptoms.

Both the dead and the living cultures are inoculated intravenously; subcutaneous injection gives rise to abscesses.

An interval of a fortnight is allowed to elapse between the last dose and the bleeding, which is carried out in the usual way.

Testing the Anti-plague Serum

The protective power of the serum may be tested by injecting $\frac{1}{20}$ c.c. of the serum into a mouse, and twenty-four hours afterwards pricking it in the hind foot with a needle dipped in a plague culture. This amount of serum should suffice to protect the animal completely.

The curative power of the serum is tested by pricking a mouse in the hind foot with a needle dipped in a plague culture, and sixteen hours afterwards injecting $\frac{1}{4}$ c.c. of the serum; this should be sufficient to save the animal's life.

The preparation of the cultures, inoculation of the horses, and testing of the serum must be carried out with all precautions to avoid the dissemination of infection. The access of flies to the cultures or test animals should be prevented by the use of wire gauze.

Lustig's Anti-plague Serum

Lustig and Galeotti have devised an anti-plague vaccine; it is prepared by growing the bacillus on the surface of agar in dishes for three days, scraping off the growth, and treating with 1 per cent. caustic soda solution. The fluid is then filtered through paper and precipitated with very dilute acetic or hydrochloric acid or by saturation with ammonium sulphate. The precipitate is dissolved in a 0·5 per cent. solution of sodium carbonate, and filtered through a Chamberland filter. This forms the vaccine fluid, which has the chemical characters of a solution of nucleo-proteids. By repeated inoculation of horses with this vaccine, an anti-serum can be obtained.

Therapeutic Use of Anti-plague Serum

It is difficult to estimate with any approach to accuracy the value of anti-plague serum, on account of the variation of the mortality of the disease in the different clinical varieties, in different epidemics, and in different races, and the influence of sex and age. Choksy gives the following summary of the mortality:

'The mortality rates in the principal types of plague may be put down as follows:

	Mortality
Simple bubonic plague	77·25 per cent.
Septicæmic plague	89·62 ,,
Pneumonic plague	96·69 ,,
Cellulo-cutaneous plague . . .	62·00 ,,

'The average mortality in plague has not been what it is to-day. During the epidemic of 1896–97 it stood at about 61·53 per cent. at the Arthur Road Hospital, and 64·5 per cent. and 68·28 per cent. at the Government House and Grant Road Hospitals, respectively. The second epidemic of 1897–98 showed a higher rate, from 78·55 per cent. at the Arthur Road Hospital to 79·26 per cent. at the Grant Road Hospital. The third epidemic of 1898–99 gave a still higher rate, the lowest being 78·97 per cent. at Arthur Road Hospital, and the highest 81·40 per cent. at the Modikhana Hospital. The average mortality in 5,836 cases treated at the Modikhana, Maratha, and Arthur Road Hospitals during 1898–99 was 80·39 per cent. During the fourth epidemic of 1899–1900 the Maratha Hospital shows a mortality of 80·95 per cent. in

2,599 cases, and the non-serum cases at the Arthur Road Hospital have a mortality of 79·54 per cent. So that, for all practical purposes, the normal mortality rate of plague in our *public hospitals* may safely be put down at 80 per cent. The influence of race, age, and sex may be gathered from the following data :

Race	Mortality rate
Europeans	30 to 40 per cent.
Eurasians	35 ,, 45 ,,
Parsees	45 ,, 55 ,,
Mahomedans (higher classes) . .	50 ,, 60 ,,
,, (lower classes) . .	60 ,, 65 ,,
Native Christians (Goanese) . .	60 ,, 65 ,,
Hindus (high caste)	65 ,, 70 ,,
,, (low caste)	75 ,, 85 ,,

'An analysis of 6,000 hospital cases gives the following mortality rates according to sex and age amongst Hindus, Mahomedans, and native Christians. More than 5,000 of them were Hindus :

—	Males	Females	Children	Total
Hindus	79·71	77·47	69·57	75·33
Mahomedans . . .	64·76	78·04	58·82	65·73
Native Christians . .	63·77	68·36	58·97	64·34

'The mortality in males of all the races is the highest, and in children the lowest, the difference varying from 5 to 10 per cent. between them. The mortality in Mahomedan and Christian females is apparently higher than in males, because of their smaller number.'

Symmers prepared anti-plague serum, which he believed to be as potent as Yersin's, but experimentally it possessed little immunising or curative power. On the other hand, Calmette and Salimbeni report favourably upon the use of Roux's serum in the epidemic of plague at Oporto. Of 63 cases treated before serum was available, 18 went into hospital and 45 were treated at home; of the former 7 died, of the latter 21 died, giving a case-mortality of 39·0 and of 46·6 per cent. respectively. From September 3 to November 18, 142 cases were treated with the serum in hospital, of whom only 21 died, a case-mortality of 14·8 per cent. During the same period, of 72 cases in their own homes, and not treated with the serum, 46 died, a case-mortality of 63·7 per cent.

Choksy treated 480 cases with Lustig's serum, of whom 328 died, and 152 recovered, a case-mortality of 68·3 per cent.; while of a similar number of cases not treated with the serum, 382 died and 98 recovered, a case-mortality of 79·6 per cent. Eliminating moribund and convalescent cases, the case-mortalities come out at 60·4 per cent. for the serum, and 79·8 per cent. for the non-serum, cases.

Clemow has used both Yersin's and Lustig's sera, and does not report favourably upon either. But the cases were few and the dosage small.

Serum treatment in India does not seem to have been very successful, and the Indian Plague Commission has not reported favourably upon it. The writer believes however that this want of success is partly due to insufficient doses (see below).

Dosage and administration.—Anti-plague serum does not seem to be very potent, and the non-success of the treatment in India is probably to some extent due to the use of too small an amount. Calmette lays down the following rule for the administration of the serum: All patients suffering from the bubonic or pulmonary form of plague should be treated as soon as possible, and should receive at once 20 c.c. by *intra-venous* inoculation, followed by two subcutaneous injections of *at least* 40 c.c. each within the first twenty-four hours. Afterwards 10 c.c., 20 c.c., or 40 c.c., according to the condition of the patient, should be given daily subcutaneously until the temperature has become normal.

Choksy lays down the following rules for the administration of Lustig's serum :—

‘1. As soon as the diagnosis has been made, inject 60 to 80 to 100 c.c. in adults; for children under twelve, half the dose; for infants, 10 c.c. *The patient must be injected as early as possible. Much valuable time is lost in waiting.*’

It would be well to give a first injection of 20 to 30 c.c. intravenously as recommended by Calmette.

‘2. Injections should be given in the morning and repeated after twenty-four hours. The patient, if seen for the first time in the afternoon or evening, should be injected at once, and the next injection should be given in the following morning.

‘3. The quantity to be injected on subsequent occasons should depend upon the range of the temperature on the previous evening and the general condition of the patient. If the temperature is the same as on the evening of the day of the first injection, 40 to 60 c.c. may again be injected; if lower, then 30 c.c. or less.

‘4. The quantity of the serum injected should be gradually decreased day after day, as above, until the temperature reaches to normal in the morning.

‘5. There is a drop in temperature of one to three degrees or more during the course of plague, and it may occur on any day from the second to the seventh. The injections should not be discontinued when this happens.

‘6. Injections in the evening are not necessary, unless secondary buboes develop, or the temperature suddenly goes up higher than on the previous evening; 30 to 40 c.c. may be injected under these circumstances.

‘7. If the temperature on any evening is found to be lower than in the morning, it is a favourable indication, and the quantity of serum injected the following morning may be safely reduced.

‘8. Six to eight injections may be required to effect a cure.

‘9. The total quantity required for a cure may vary from 150 to 300 c.c., depending upon the severity of the case, complications, &c., and the strength of the serum used.’

In one very bad case Choksy states that he administered 240 c.c. in four doses of 60 c.c., each within thirty-six hours, with the happiest results. In a case of accidental infection during the epidemic at San Francisco, with the development of plague pneumonia, which was treated with anti-plague serum, 60 c.c. were injected subcutaneously, and the same quantity intravenously, the whole amount being given within twenty-four hours. Within a few hours after the last injection the temperature dropped to 100°, and before the end of the third day reached the normal.

Martini, as the result of an experimental study of plague, concludes that 100 c.c. of anti-plague serum should be given subcutaneously for a dose. In plague pneumonia, 50 c.c. should be administered intravenously, and 50 c.c. subcutaneously.

Cairns gives the following as his conclusions as to the value of anti-plague serum based on cases treated during the Glasgow epidemic of 1901 : (1) Yersin's serum is a remedy of the greatest value in the treatment of bubonic plague ; (2) its action is bactericidal as well as anti-toxic ; (3) this double action of the serum is best secured by its early administration in large doses, both subcutaneously into the lymphatic area which drains towards the bubo and also intravenously ; (4) in mild cases the former will suffice, but in severe cases the combined method should be employed. For the latter the initial combined dose should be 150–200 c.c.

The writer would therefore urge that, if serum treatment be adopted in plague, the serum should be administered in large doses.

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Injection of Anti-streptococcic and Anti-plague Sera

Denys and Tartakowsky state that the efficacy of anti-streptococcic serum is considerably enhanced if it be injected into the neighbourhood of the infective focus, in such a manner that the serum is carried by the lymphatic vessels towards this focus.

The same seems to hold good for anti-plague serum. If a guinea-pig be infected with plague intraperitoneally, and if the exudate be examined a short time afterwards, no phagocytosis will be observed and the animal ultimately succumbs. The same is the case if the animal be injected subcutaneously with anti-plague serum, but if the serum be injected intraperitoneally phagocytosis will be observed to take place and the animal survives. In this manner, 0·1 c.c. of the serum injected intraperitoneally may save the animal, whereas 10 c.c. subcutaneously may fail. Similarly, if the guinea-pig be infected in the foot, a great difference is observed whether the serum be injected in the foot or in the back. In the first instance the local lesion becomes much less pronounced and the buboes are limited. The authors consider that these results are applicable to treatment in man.[1]

Enteric Fever

The typhoid bacillus under ordinary conditions produces little or no toxin in artificial culture media, and all attempts to produce an anti-toxic serum by injection of the dead or living microbes have hitherto proved failures. Chantemesse, however, by a special method of cultivation, claims to have been able to prepare a soluble typhoid toxin, and with this, by inoculating horses, to have obtained an anti-toxic curative serum. The culture-medium consists of a maceration of spleen and bone marrow with some defibrinated human blood. From January 1 to October 10, 1901, 371 typhoid patients were treated by ordinary methods in the principal Paris hospitals, with 109 deaths, a case-mortality of 29 per cent. During the same period 100 cases were treated with the anti-toxic serum of Chantemesse. Of these, 6 only died, *i.e.* a case-mortality of 6 per cent. All those who had been treated within the first ten days of the illness recovered. The charts accompanying the paper show that the injection of the serum exerted a marked influence upon the temperature. In some instances within a day or so the temperature fell nearly to normal and convalescence was partially established. In others there was a fall in the temperature for two or three days, followed by a rise, but on a second injection of serum the temperature fell permanently either suddenly or gradually. The first dose of serum was 10–12 c.c.; at the end of eight or ten days if defervescence was not complete, a second dose of 4–10 c.c. was given, the amount depending upon the height of the temperature.

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[1] *La Semaine Médicale*, January 31, 1900, p. 40.

In a later paper, Chantemesse gives further results obtained with his serum. Of 1,192 cases of typhoid fever treated in the Paris hospitals from April 1 to December 1, 1902, 286 died, giving a case-mortality of nearly 24 per cent.

During the same period, 179 cases were treated with the serum, of whom 7 died, a case-mortality of only 3·7 per cent. Chantemesse is careful to point out that results based on a small number of cases may be most unreliable. For example, at the Laennec Hospital from April 1 until December 31, 1901, there were 44 cases of typhoid fever without a single death, whereas in the following year, of 42 cases 11 died, a mortality of over 25 per cent.

The ordinary anti-typhoid sera on the market are anti-microbic sera obtained by treating a horse first with killed and afterwards with living cultures of a virulent typhoid bacillus, just as for the anti-plague serum. This anti-typhoid serum has given disappointing results. Thus 18 cases of enteric fever were treated by Newall with anti-typhoid serum procured from 'a reliable source.' The dose used was either 5 c.c. or 10 c.c. injected into the iliac region; in some cases only one injection was given, in others two, three, or four were administered. Some cases were treated with serum alone, others had intestinal antiseptics, and symptomatic treatment of complications was adopted. It may be said that in none of these cases (nor in 13 others subsequently treated) did the use of the serum appear to have any beneficial effect. In 4 cases the temperature fell markedly after the first dose for a short time, but with subsequent doses not at all.

Macfadyen has recently used the intracellular constituents of the typhoid bacillus, obtained by triturating the bacillus with liquid air, for immunising apes, and claims that an anti-toxic serum is thereby obtained. This method is, however, at present only in the experimental stage.

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Anti-typhoid Extract of Jez

This is prepared by injecting rabbits with typhoid culture and so immunising them, killing the animals, and then extracting the minced-up spleen, brain and spinal cord, bone-marrow, and thymus gland with a solution consisting of sodium chloride, glycerine, and alcohol, with a little carbolic acid. It forms a dark, reddish-yellow fluid of alkaline reaction which is administered by the mouth. According to the severity of the case, a dessert-spoonful is given every one or two hours until the temperature becomes remittent, then every three hours until the morning temperature does not exceed 100·5° F. The total amount of the extract required by a patient averages 17 fluid ounces. Under the treatment, the general condition is said to improve, the pulse becomes slower, and the temperature generally falls considerably, and the morning remissions become more marked.

See Jez, *Wien. klin. Woch.*, January 24, 1901. Ref. in *Brit. Med. Journ.*, Epit., 1901, i. p. 51, No. 212, and *ib.*, 1902, i. p. 27, No. 108.

Other Diseases which have been treated with Anti-sera

There is some reason to think that the serum treatment of disease has been carried to excess; in fact, there seems to be hardly any condition for which an anti-serum has not at some time or other been tried.

Care must be taken not to confuse the various vaccines (anti-typhoid, anti-cholera, anti-plague, &c.) with the anti-sera.

The following are some examples of other diseases, arranged in alphabetical order, in which anti-sera have been employed.

Anthrax

Sclavo, Marchoux, Sobernheim (see p. 38), and others have prepared anti-sera against anthrax.

Lazaretti reports 23 cases of anthrax in man treated with Sclavo's serum with only 1 death.

San Felice has immunised dogs against anthrax, and with their serum has treated a case of anthrax in man.

Lazaretti, *Brit. Med. Journ.*, Epit., 1902, ii. p. 64; Marchoux, *Ann. de l'Inst. Pasteur*, ix. 1895, p. 785; San Felice, *Centr. f. Bakt.* Abt. i., *Originale*, xxxiii. 1903, p. 61; Sobernheim, *Brit. Med. Journ.*, Epit., 1902, ii. p. 90.

Botulism (Meat and Sausage Poisoning)

One form of poisoning, arising from the ingestion of unwholesome meat and sausages, is due to infection with an anaërobic bacillus, the *B. botulinus* of Van Ermengem. This organism produces a powerful toxin with which animals may be immunised, and a truly anti-toxic serum obtained.

See Van Ermengem, *Ann. de Micrographie*, viii. 1896, p. 66; Kempner, *Zeitschr. f. Hyg.*, xxvi. p. 481 and xxvii. p. 213.

Cholera Asiatica

No satisfactory anti-cholera serum has yet been prepared. A serum obtained by injection of animals with increasing doses of cholera cultures is of little or no value. Metschnikoff, Roux, and Salimbeni, by growing the cholera vibrio in a gelatin peptone-water salt solution in a shallow layer for a few days, obtained a feeble toxin, with which they were able to immunise animals and to obtain an anti-toxin which experimentally possessed curative properties, but which does not seem to have been used in treatment. The prospect of preparing an anti-cholera serum does not seem to be very hopeful, and even if prepared, it would have but a limited use, since the disease runs such a rapid course.

See *Ann. de l'Inst. Pasteur*, x., 1896, p. 257.

Infection by the Bacillus Coli

The *Bacillus coli* plays an important rôle in human pathology, causing the peritonitis following perforation and obstruction of the bowel, certain forms of puerperal infection, ischio-rectal abscess, cystitis and pyelitis, &c. Albarran and Moser have prepared an anti-serum by injecting animals with cultures, and suggest that it might be used in infections of the urinary tract, or before operations upon the urinary organs, to prevent subsequent infection.

See *Journ. Amer. Med. Assoc.*, January 17, 1899.

Dysentery

An attempt has been made by Shiga to prepare an anti-dysenteric serum for use in the bacillary form of dysentery by treating a horse with cultures of the *bacillus dysenteriæ* (Shiga and Flexner), and with the serum he claims to have considerably reduced the mortality in the epidemic dysentery of Japan.

Celli in Italy has also prepared an anti-dysenteric serum by means of the *bacillus coli dysentericus.*

See Flexner, *Bull. Johns Hopkins Hosp.*, xi., 1900, p. 231; Kruse, *Deutsch. med. Woch.*, 1903, No. 1, p. 6, No. 3, p. 49; Valagussa, *Annali d'Igiene Sperimentale*, x. fasc. iv., 1900.

Anti-thyroid Serum for Exophthalmic Goitre

Acting upon the supposition that the secretion of the normal thyroid gland is used to neutralise a toxin present in the body, and that an over-production of this neutralising secretion causes an intoxication known as exophthalmic goitre, Ballet and Enriquez have used in the treatment of the disease the serum of dogs whose thyroids had been previously extirpated and which, therefore, on the above supposition, would contain the toxin, which on injection would neutralise the excess of thyroid secretion. Several cases have now been reported as benefited by this treatment. The serum termed ‘anti-thyroidin’ is supplied by Messrs. Merck.

See Ballet and Enriquez; Schultes, *Münch. med. Woch.*, May 20, 1902, and *Brit. Med. Journ.*, Epit., 1902, ii. p. 71; *Lancet*, 1902, i.

Hay Fever

Hay fever, which is universally admitted to be produced by the action of the pollen of various species of *gramminaceæ*, from the experiments of Dunbar seems to be caused not by the mechanical irritation of the pollen grains, but by a toxic substance contained in them. By extracting this toxic substance, which possesses intensely irritating properties upon the nasal mucous membrane, by means of ether, and injecting the ethereal extract into horses, Dunbar has obtained an anti-serum which, from his own experiments and those of Semon, immediately causes the disappearance of the subjective symptoms produced by applying the toxin to the conjunctiva and may prove of use in the treatment of this troublesome complaint.

See Semon, *Brit. Med. Journ.*, 1903, i. p. 713.

Hydrophobia

Tizzoni and Centanni have prepared an anti-rabic serum by immunising sheep with gradually increasing doses of anti-rabic vaccine (prepared according to the Pasteurian method). The serum is stated to be both protective and curative. The ordinary method of inoculating for rabies or hydrophobia is useless if the disease has declared itself: it is only a preventive.

See *Lancet*, 1895, ii. p. 659 *et seq.*

Leprosy

Carasquilla has prepared a serum for leprosy by injecting asses with the blood derived from advanced cases of the disease. Herman and Abraham have immunised horses with the juice obtained from the leprous nodules and emulsified in saline solution. Anti-venene has also been extolled in the treatment of this disease by Dyer, of New Orleans, but in the writer's hands utterly failed to produce any improvement in a well-marked case, as did also diphtheria antitoxin (as was expected).

See *Centr. f. Bakt.* Abt. i., xxiv. pp. 178 and 179.

Malignant Disease

An attack of erysipelas having a beneficial action in cases of carcinoma, it was proposed to employ an anti-erysipelatous serum in the treatment of this condition, and at one time encouraging reports were published of this mode of treatment. No lasting good, however, seems to result, and the treatment has fallen into disrepute, and it is difficult to conceive that it should have had any effect.

No form of serum treatment has yet been devised for malignant disease, and the chief hope for the future would seem to lie in the preparation of an anti-cellular serum by injecting an animal with macerated carcinoma and sarcoma tissues. Good effects are stated by Jensen to be obtained in the carcinoma of white mice by treating them with a serum obtained from an animal injected with the white-mouse carcinoma.

Leyden and Blumenthal tried the same method on dogs. Carcinomatous tumours from dogs were extirpated, divided as finely as possible, and injected subcutaneously for many weeks into rabbits. The rabbits' serum was then used to treat dogs suffering from carcinoma with good results. They then applied these results to man, the same method being adopted with human carcinomatous tumours in order to prepare a serum. This serum was used in some inoperable cases with decided benefit.

Hoyten has used the juice expressed from carcinoma in the treatment of inoperable cases with apparent benefit. Ballance and Shattock, however, did not obtain any appreciable beneficial effect in cases of carcinoma in man by the use of the serum of a horse injected with glycerin extracts of carcinomatous tumours. (See also Coley's Fluid, p. 252, and Cancroin, p. 254.)

See Leyden and Blumenthal, *Deutsche med. Woch.*, September 4, 1902, and *Brit. Med. Journ.*, Epit., 1902, ii. p. 59; Hoyten, *Brit. Med. Journ.*, 1902, ii. 1342.

Malaria

Plehn has used an 'anti-horse sickness serum' in the treatment of malaria.

Malta Fever

Wright and Semple describe a case of Malta fever treated with an anti-serum. No details are given of the preparation of the serum, but presumably the method is the same as for any other anti-serum.

See *Lancet*, 1899, i. p. 1024.

Pernicious Anæmia

Hunter has treated a case of pernicious anæmia with anti-streptococcic serum, apparently with benefit.

See *Lancet*, 1901, i. p. 473.

Acute and Chronic Rheumatism

Menzer concludes, as the result of his studies, that rheumatism is the result of a streptococcic infection. He has immunised horses with ascitic bouillon cultures of a streptococcus obtained from the tonsils of a rheumatic-fever patient. The effect of the serum from animals so immunised is not exactly curative, for the inflammatory condition of the joints is at first aggravated, but this is followed by absorption and rapid convalescence.

He gives 5–10 c.c. of the serum daily, and 50–75 c.c. altogether. Normal serum and Marmorek's anti-streptococcic serum had no effect.

See *Treatment*, vi., 1902, p. 556, and *Brit. Med. Journ.*, Epit., 1902, ii. p. 47.

Scarlatina

As the micro-organism of scarlatina is not known with certainty, the treatment of the disease with an anti-serum must be more or less empirical. At the same time there is some amount of evidence to show that this disease is due to a species of streptococcus, and the anti-sera that have been used have been prepared with some strain of this organism. Ordinary anti-streptococcic serum has been employed with benefit in certain cases, especially where there has been an acute condition of the throat. Moser, having isolated a streptococcus from the heart-blood, immunised animals with it, and so obtained an anti-serum with which he has treated 84 cases with apparent benefit, the dose being from 30 to 180 c.c.

An anti-streptococcic serum prepared by Dr. Hubbert, of the firm of F. Stearns & Co., of Detroit and Windsor, was used by Charlton in the treatment of 15 severe cases of scarlatina, all of which would have had a lingering convalescence, or might have terminated fatally. Of these, 13 made a prompt recovery without complications, the other two died. The dose of serum given was 20 c.c., which was repeated once in the most severe cases. The effects of the injections were (1) rapid subsidence of the pyrexia, (2) decrease in pulse rate with improvement in tension and rhythm, (3) prevention or amelioration of cervical adenitis, otitis, and albuminuria, (4) rapid and favourable convalescence.

Moser, *Wien. klin. Woch.*, October 9, 1902; Charlton, *Montreal Med. Journ.*, October 1902, p. 753.

Anti-staphylococcic Serum

Attempts have been made to prepare a serum which will antagonise the effects of the *staphylococcus pyogenes aureus*, the commonest of the pyogenic cocci, but so far without much success. Doyen claims to have obtained a satisfactory serum with which he has treated cases of furuncle, phlebitis, &c. with benefit. The amount injected was 5 c.c. The serum was active against the *S. pyogenes aureus*, but not against the streptococcus. Paltchikowsky has also immunised horses by means of the *S. pyogenes aureus*, and has obtained a serum which would protect against the *S. aureus* or *albus*. Pröscher points out that therapeutically it is important to know whether the various strains of staphylococci are identical. By the agglutination test, it was found that staphylococci of pus were agglutinated by the serum of the patient, but those from the air, skin, and vaccine were not so agglutinated, pointing to a difference between the races. By inoculating rabbits subcutaneously for a month, a serum was obtained of which 1 c.c. would protect against 5–7 times the minimal fatal dose. (See also p. 224.)

Doyen, *Revue de Thérapeutique*, February 15, 1903, p. 117; Paltchikowsky, Ref. in *Bull. de l'Inst. Pasteur*, i., 1903, No. 2, p. 90; Pröscher, *Deutsche med. Wochenschr.*, 1903, No. 11.

Syphilis

Attempts have been made to prepare an anti-syphilitic serum by injecting animals with the blood of syphilitic patients, also with micro-organisms alleged to have been isolated from cases of the disease. Since the specific organism of syphilis is not known with certainty, these sera cannot be regarded as having any value.

Trypanosomiasis

Laveran has found that in certain cases human serum has an inhibitory action in cases of nagana (tsetse fly disease), and it has been suggested therefore that horse serum might be of use in cases of human trypanosomiasis. Attempts to immunise against trypanosomata have proved failures.

Tuberculosis

Numerous attempts have been made to prepare a serum or anti-toxin for the treatment of that scourge of civilisation—tuberculosis. The best-known sera appear to be those of Paul Paquin in America and of Maragliano in Italy. The exact details of the preparation of these sera do not seem to have been published; but the general method is to treat animals with increasing doses of tuberculin and extracts of tubercle bacilli, then with the dead bacilli themselves, and finally with living virulent bacilli. It cannot be said that, as a whole, the published results of the treatment are very promising. Mircoli publishes the statistics of the treatment of 2,899 cases of pulmonary tuberculosis in various stages with Maragliano's serum—1 c.c.

on alternate days. Of these cases, over 14 per cent. are stated to have been cured, 50 per cent. improved, and in only 18 per cent. did the disease tend to progress during treatment. Mircoli states that the blood-serum of normal individuals has an anti-toxic action towards the tubercle toxins, whereas that of tubercular patients has not, but regains this property after about a month's treatment with the serum. Paquin states that he has had 29 cases of recovery in cases of pulmonary, throat, bone, joint, kidney, and testicle tuberculosis with serum treatment exclusively.

See Mircoli, *Journ. Amer. Med. Assoc.*, 1900, October 6, pp. 887 and 914, and *Gaz. degli Ospedali*, September 9, 1900; Paquin, *Journ. Amer. Med. Assoc.*, 1900, October 27, p. 1076.

Whooping Cough

Leuriaux has isolated a bacillus from the sputum in whooping cough, and by immunising horses with filtered broth cultures claims to have obtained a serum which is efficacious in this disease. If the treatment were commenced early it is stated that the symptoms usually disappeared within a few days.

See *La Semaine Médicale*, No. 29, July 1902, and *Treatment*, vi. 1902, p. 667.

Yellow Fever

Sanarelli has claimed to have prepared an anti-serum for yellow fever by immunising animals with cultures of his *B. icteroïdes.* But since grave doubt, to say the least, has been cast on the specificity of this organism, through the work of American investigators, the yellow-fever serum must be regarded with suspicion.

See Sanarelli, *Ann. de l'Inst. Pasteur*, xii., 1898, p. 348.

Attempts have been made to utilise the blood of individuals who have suffered and have recovered from an attack of disease for the treatment of other cases of the same disease, *e.g.* in scarlatina and variola. Obviously such a procedure could have but a very limited application, even if of any value, which is extremely doubtful. The serum of vaccinated calves has been used for the treatment of variola.

Diphtheria anti-toxin has been given in asthma, 1–3 c.c. injected subcutaneously on two or three days, it is stated, with considerable benefit. Christmas has prepared an anti-gonorrhœal serum by injecting rabbits with cultures of the gonococcus. There are no reports of patients treated with this serum.

CHAPTER VI.

TRANSFUSION—TRANSFUSION OF ANIMAL BLOOD—SALINE SOLUTIONS AND ARTIFICIAL SERA—NORMAL SERUM FOR ALIMENTATION

Transfusion of Blood

The transfusion of blood has of late years fallen into discredit, for the success of the method is recognised to be due not to the corpuscular elements, but to the fluid introduced. The corpuscles of one species of animal are rapidly destroyed when introduced into another species, hence it is useless to transfuse the blood of a lower animal.

Direct or immediate transfusion, in which the blood of the donor is introduced directly into the veins of the recipient, is now never practised.

Indirect or mediate transfusion consists in bleeding a healthy or healthy individuals, receiving the blood into a bowl kept warm by standing in hot water (100° F.), whipping the blood to remove the fibrin, straining through fine muslin which has been treated with boiling water or boiled to sterilise it, after which it is injected intravenously into the patient in the manner detailed at p. 66.

Saline infusion has now almost entirely taken the place of blood transfusion. Blood transfusion should be employed only in cases of grave anæmia, and then is of doubtful value. The loss of the red corpuscles may be largely compensated for by oxygen inhalation.

Transfusion of Animal Blood as a Therapeutic Measure

The transfusion of blood from one animal into another or into man is frequently followed by dangerous symptoms, due to toxic or hæmolytic action or both. These are usually much more marked when the blood is heterogeneous, *i.e.* is derived from a species different from that into which it is injected.

The symptoms are rigors and fever, sweating, albuminuria, and hæmoglobinuria, agglutination and solution of the blood corpuscles, and enlargement of the spleen.

Bier has suggested that these phenomena might be made use of as a therapeutic measure, and has employed defibrinated lamb's blood in cases of tubercular disease and cancer. The amount injected by transfusion ranged from 4 c.c. to 20 c.c., and the transfusion was repeated only at intervals of a week or ten days. Four cases of lupus showed marked improvement, and in a case of sacro-iliac tuberculosis after twelve injections the suppuration in the pelvis had nearly ceased, the fistulæ were almost healed, the patient had gained in weight and was able to be up. The injections were followed by marked reaction. The technique of the injection is simple. An assistant lightly grasps the arm above the elbow, so as to produce venous engorgement and swelling of the superficial veins. The operator takes a swollen superficial vein near the elbow between the finger and thumb of his left hand, and, the skin being cleansed, introduces into the vein the sterile needle of a Pravaz syringe. When the needle is in the lumen of the vein, which is known by the blood welling out, the assistant attaches the filled syringe and slowly injects the defibrinated blood.

Münchener med. Wochenschr., 1901, No. 15, April 9, p. 569.

Saline Infusion

The infusion of saline solutions is a most valuable method of treating severe hæmorrhage, shock, and collapse, and for this purpose entirely replaces blood transfusion.

Various formulæ have been devised for the preparation of saline infusions, and tabloids or cachets can be obtained containing the requisite constituents for preparing a given amount of solution, and are very convenient, the object being to imitate the saline constituents of the blood. Hare gives the following formula :

Calcium chloride	0·25 gram	or	2 grains
Potassium chloride	0·10	,,	1 grain
Sodium chloride	9·0 grams	or	70 grains
Sterilised water	1000·0	,,	1 pint

A solution of 1 drachm of common salt to the pint of water does perfectly well for ordinary purposes.

If there be time, the solution may be prepared and then sterilised by boiling ; but if not, the salts should be placed in a vessel which has been scalded out, and an ounce or two of *boiling* water added to dissolve and sterilise them, the solution being then made up to the right amount by the addition of *boiled* water at a temperature of about 120° F. A small funnel is attached by a short length (18 in.) of rubber tubing to a glass or metal cannula, and the whole is sterilised by thorough rinsing with boiling water, or with an antiseptic followed by boiled water. The rubber tubing should be closed by a spring or screw clip.

The median basilic is one of the most convenient veins to expose. In opening the vein surgical cleanliness must be carried out.

After exposing the vein and incising it, a sterilised cannula is inserted into the opening, and is held in position by means of a catgut ligature.

Care must be taken that air is not admitted with the solution. This danger has been exaggerated; a bubble of air does no harm, the quantity to cause death must be considerable. To avoid the injection of air with the solution, allow the fluid to run through the cannula while the latter is being inserted into the vein.

In hæmorrhage or shock from other causes the amount of fluid to be injected in an adult should never be less than a pint, frequently two to four pints are required. Of course, the quantity is determined by the patient's general condition.

The fluid should be injected at the rate of a pint in fifteen minutes, and at a temperature ranging from 105° to 110° F. It is remarkable how rapidly a solution at this temperature will stimulate a flagging heart. When the pulse comes down to 120, or thereabouts, the cannula may be removed, the vein ligated and the incision closed. If it is necessary to repeat the dose, a hot saline rectal injection, as a rule, will give good results, or the solution may be injected into the cellular tissue. In collapse,[1] *e.g.* in severe vomiting or diarrhœa or in cholera, large amounts of fluid must be injected.

These saline solutions must always be sterilised before use, as they possess no inhibitory properties against the development and multiplication of micro-organisms. To remedy this Tavel suggested the addition of a small quantity of sodium carbonate (0·1 per cent.); but Baisch[2] has found that this induces gangrene when injected subcutaneously.

Barker[3] recommends the subcutaneous injection into the axillary region of 500 c.c. of a solution composed of 5 grams of glucose dissolved in 100 c.c. of 0·6 per cent. salt solution twice daily in exhausted patients for some days preceding and following operation.

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[1] It is to be noted that the pathology of shock and of collapse are different. In shock the vessels in the splanchnic area—*i.e.* the abdominal veins—are enormously dilated, and the patient bleeds, as it were, into his own vessels. In collapse there is withdrawal of fluid from the blood and tissues.

[2] *Brit. Med. Journ.*, Epit., 1902, ii. p. 67, No. 276.

[3] *Brit. Med. Journ.*, 1902, i. p. 770.

'Serum' of Trunecek

This is a saline solution which is inoculated subcutaneously and is stated to be of great benefit in arterio-sclerosis. The salts employed are

Sodium chloride	10 grams
Sodium sulphate	1 gram
Calcium phosphate	0·75 gram
Magnesium phosphate	0·75 ,,
Sodium carbonate	0·40 ,,
Sodium phosphate	0·30 ,,

One gram of this mixture is dissolved in 15 c.c. of sterile distilled water. The treatment is commenced by injecting hypodermically, preferably into the region of the buttock, 2 c.c. of this sterile solution, and the injection is repeated every other day, being increased in amount by 1 c.c. on each occasion, until a dose of 8 c.c. is reached. In special cases the dose may be increased to 12 c.c. The mixture has also been given *per rectum* and by the mouth.

See *La Presse Médicale*, January 15, 1902, and June 18, 1902; *Le Nord Médical*, November 1, 1902, p. lxxxi. Also *Treatment*, vol. vi., 1902, pp. 267 and 417.

Blood-serum Injections for Alimentation

Sterile blood-serum, preferably horse serum that has been heated to 60°–62° C. for half an hour to destroy the rash- and fever-producing constituents, may be administered by subcutaneous injection in cases where food cannot be given by the stomach on account of vomiting, obstruction, &c., or may be used to supplement gastric or rectal feeding.

Salter has found that rats and mice may be kept alive for weeks by means of injections of serum alone without any other food.

The doses given must, however, be adequate: to young children 30–50 c.c., to adults 100–150 c.c. may be given daily. In a case of gastric carcinoma the writer supplemented gastric feeding with injections of serum with excellent results.

Kirton has used subcutaneous injections of sterile horse serum when mouth, nasal, and rectal methods of alimentation failed, and speaks favourably of them. He recommends 20–40 c.c. subcutaneously, daily.

Injections of ox serum have been used in the treatment of chorea.

See Salter, *Guy's Hospital Reports*, liii.; Kirton, *Lancet*, 1901, i. p. 1666; *Brit. Med. Journ.*, Epit., 1902, i. p. 36, No. 147 (Chorea).

CHAPTER VII.

TUBERCULINS—FILTERED TUBERCLE CULTURES—MALLEIN

Tuberculin

Two varieties of tuberculin must be described, the 'old tuberculin' and the 'new tuberculin,' both first prepared by Koch.

The *old tuberculin*, introduced as long ago as 1890, consists essentially of a boiled and filtered broth culture of the tubercle bacillus. The mode of preparation is briefly as follows. The tubercle bacillus is grown in a glycerin veal broth for six to twelve weeks in a shallow layer in flat flasks (fig. 20), so that there is a free supply of

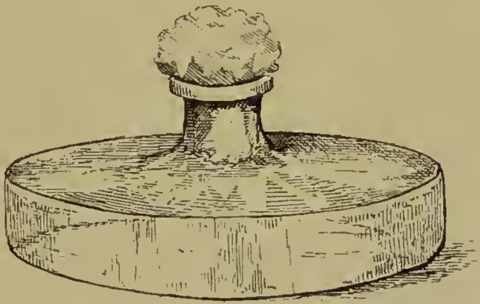

Fig. 20.—Flask for growing Tuberculin.

oxygen and an abundant growth with copious film formation. The latter seems to be essential, but it does not appear to matter whether the bacilli are virulent or non-virulent. The cultures, bacilli and all, are concentrated over a water bath to about one-tenth of their volume, and then filtered through porous porcelain ; the resulting fluid is thick, owing to the concentration of the glycerin by the evaporation, of a dark amber colour, and possesses a curious characteristic smell. The large proportion of glycerin preserves the fluid, which keeps indefinitely in a cool dark place.

It is perhaps preferable first to concentrate to one half over the water bath, then to filter through a Chamberland filter, and, after filtration, to concentrate further over the water bath until the fluid is reduced to one-tenth of the original volume. If first concentrated to one-tenth, filtration is slow and there is considerable loss owing to the thick nature of the fluid.

So prepared, the old tuberculin possesses remarkable properties. Injected into a healthy animal or individual it produces no effect, but in a tubercular one minute doses, 0·0003 c.c., give rise to a marked reaction—elevation of temperature with constitutional disturbance more or less severe, and swelling and tumefaction of tubercular lesions (glands, ulcers, &c.). By cautiously increasing the dose a toleration is gradually induced, so that large doses cause little or no disturbance. Under certain conditions the injections of tuberculin produce marked changes in the tubercular parts, leading to necrosis and exfoliation, with subsequent healthy reaction and repair; this is especially seen in cases of lupus. By continued injections a marvellous improvement results, so much so that a cure is apparently effected; but unfortunately when the tuberculin treatment is discontinued the scar usually breaks down and the disease returns. Nevertheless some cases remain permanently healed.

Healthy guinea-pigs bear considerable injections of tuberculin without harm; but if they be tubercular, doses of 0·01 gram produce death if the disease is advanced (eight or ten weeks after inoculation); if less advanced (four to five weeks after inoculation), a larger dose, 0·2 to 0·3 gram, is required; but 0·5 gram always proves fatal. There is no method of accurately standardising tuberculin: attempts have been made to do so by intracerebral injection, but have not proved successful. A rough standardisation may be effected by injecting guinea-pigs; healthy animals should be able to withstand an injection of

1 c.c. without harm, tubercular ones inoculated six weeks previously with a pure culture, should be killed by a dose of 0·1 c.c.

The *new tuberculin*, introduced in 1897, differs from the old by being prepared with *virulent* cultures and by being *unboiled*. It consists essentially of a solution or emulsion of the tubercle bacilli themselves.

Young cultures of a virulent tubercle bacillus are used. The bacillus may be grown on glycerin serum and the resulting growth scraped off, desiccated *in vacuo*, and then triturated. This is a dangerous process and can only be done with safety by machinery. The dry masses of bacilli are introduced into steel vessels containing unglazed porcelain balls, these are closed and kept in movement for five or six days; the continual movement of the porcelain balls grinds up the tubercle bacilli. The triturated bacilli are then emulsified in distilled water and the emulsion is centrifugalised for 30–45 minutes at a speed of 4,000 revolutions per minute. The upper slightly opalescent layer, termed tuberculin O (O = obere = upper), is pipetted off, and the residue of powdered bacilli again dried, triturated, emulsified and centrifugalised. The upper layer is the new tuberculin R (R = residual). The residue is again similarly treated and a second fraction of tuberculin R obtained, and further fractions are obtained by the same process until the residue is entirely used up. To get rid of the bacilli, the fluids are mixed and filtered through a Chamberland filter, and, in order to preserve it, 2 per cent. of glycerin is added. The fluid should contain 10 milligrams of solid matter per cubic centimetre.

The tuberculin R is the one employed therapeutically. It is stated to possess powerful immunising properties, produces little reaction in small doses in a tubercular individual, and causes no local irritation at the seat of inoculation. The tuberculin O, the fluid from the first centrifugalisation, much resembles the old tuberculin.

A third new tuberculin, tuberculin A (A = alkaline), obtained by emulsifying tubercle bacilli in a 10 per cent. solution of caustic soda, was first prepared, but since it invariably caused local abscesses, its use was given up, and the tuberculin R substituted.

Therapeutic Use of Tuberculins

1. *The Old Tuberculin*

For use, the concentrated fluid is diluted with 0·5 per cent. aqueous carbolic acid, only so much of the dilution being prepared as can be used in a few days. Half per cent., 1 per cent., 5 per cent., and 10 per cent. solutions are the most convenient, used in succession as the treatment progresses and the doses increase. The dilute solution is administered by subcutaneous injections by means of a 1 c.c. or 2 c.c. syringe, similar to an anti-toxin syringe, or with a Koch syringe (see p. 64); each should be graduated into tenths. The syringe and skin may be disinfected with absolute alcohol. The best seat for the injections is in the back, between the scapulæ. The writer has given some thousands of injections without any local trouble at the seat of inoculation.

The following general directions may be given as to the dosage.

The doses refer to the concentrated (i.e. *undiluted*) *fluid:*

(*a*) The maximum initial dose for adults is 0·001 c.c. For children and in cases of visceral tuberculosis one-half to one-tenth of this amount should be given.

(*b*) In no case should the dose be repeated until the temperature has completely fallen to normal.

(*c*) If a severe reaction occurs—*i.e.* a rise of temperature of 3·5° F. and upwards—the dose should not be increased until the reaction produced by it does not exceed about 2° F.

(*d*) At the commencement of treatment, and when the dose is increased, at least a day should intervene between subsequent doses.

(*e*) The dose should be increased at first by 0·001 c.c. until 0·005 c.c. is reached; it can then be increased by 0·002 c.c., and from 0·01 c.c. a more rapid increase is permissible, the guide being the amount of reaction, temperature, &c. produced.

(*f*) In cases of lupus the greatest amelioration is obtained by increasing the dose until 0·1 c.c. is reached, and the doses may be repeated three times a day.

The method of dosage calculated to attain the best results is somewhat disputed. In the early days of the treatment, Watson Cheyne purposely used doses sufficiently large to obtain a distinct reaction, and the following illustrates the dosage employed by him in a case of lupus:

(The small figures below the line indicate the interval in days between the doses; if there is no figure, the tuberculin was given on successive days; when the doses are included in brackets, it indicates that all were given on one day; the dashes indicate the reaction: one dash, a mild reaction; two dashes, a distinct reaction; no dash, no reaction.)

·002″ ; $_1$ ·002″ ; $_2$ ·002″ ; $_1$ ·002″ ; $_1$ ·002″ ; $_2$ ·002′ ; ·002 ; ·002 ; ·002 ; (·002, ·002) ; (·002, ·002) ; (·002, ·002) ; (·002, ·002, ·002) ; (·002, ·002, ·002) ; (·002, ·002′, ·002) ; (·004, ·004′) ; (·004, ·004) ; (·004, ·004, ·004) ; (·004, ·004) ; (·006, ·006) ; (·006, ·006) ; (·006, ·006) ; (·006, ·006) ; (·008, ·008) ; (·008, ·008, ·008) ; (·01, ·01, ·01) ; $_1$ (·015, ·015) ; (·02, ·02) ; (·03, ·03) ; (·04, ·04) ; (·05, ·05) ; (·06, ·06) ; (·07, ·07) ; (·08, ·09) ; (·09, ·1) ; ·1 ; $_1$ (·1, ·1) ; (·1, ·1) ; (·1, ·1) ; (·1, ·1) ; (·1, ·1) ; (·1, ·1) ; (·1, ·12) ; (·12, ·12) ; (·12, ·12) ; (·12, ·15) ; (·15, ·15). From this time onwards, the patient received two doses daily, gradually increasing the dose up to 0·2 c.c.

Cheyne remarks on this case that ‘there was no serious general disturbance, but the local reaction was marked and for several weeks the seat of the disease was red and scaling.’ The result was ‘very remarkable improvement with disappearance of lupus tissue.’

It is questionable whether this method of dosage is the best. The object of the injections is partly to create an immunity and by continually producing a reaction, it may well be that the *diminished* immunity which probably immediately follows an injection may be increased if successive injections be given at too short an interval and in too great an amount (see p. 224).

In all probability, especially in visceral tuberculosis, it would be better to give smaller doses, so as not to produce much reaction; this method is recommended by Goetsch who proceeds as follows:

The cases must be uncomplicated ones, and especially must be free from fever. Should the latter be present, it must be controlled and reduced by rest in bed, wet-packing, &c.

Those patients in whom the physical signs are pronounced are injected with 0·0001 gram of Koch's old tuberculin. Should this produce a rise of temperature, the dose is lowered to 0·00001 gram; and if the latter even is not well borne, the treatment is begun with the *new* tuberculin (TR) in doses commencing with 0·001 milligram and going up to 0·1 milligram. When this is reached, treatment is continued with the *old* tuberculin again, starting with 0·0001 to 0·001 gram, according to previous experience. The tuberculin is administered every second or third day, the dose being gradually and steadily increased, without producing a temperature reaction. Should this occur, the next dose is reduced. The treatment is continued until a dose of 1·0 gram of the old tuberculin is reached.

Of 175 cases treated since 1891 for four weeks or longer, 125, or 71 per cent., appeared to be cured.

The New Tuberculin. Tuberculin R

The *new tuberculin*, of which only tuberculin R (T R) is employed, is used solely for treatment and not for diagnostic purposes. The fluid is supplied by Meister, Lucius, and Brüning of Höchst-on-Maine, and the following are the directions for use issued with it:

THE NEW TUBERCULIN KOCH

Directions for use

The New Tuberculin is supplied in liquid condition. It is an opalescent liquid similar in appearance to the mixture of 5 or 6 drops of milk to half an ounce of water. It must be kept in a cool, dark, and dry store.

The solution contains 10 milligrams of solid substance in each cubic centimetre.

The treatment is generally commenced with $\frac{1}{500}$ of a milligram of solid substance. If a reaction appears, the dose must be still further reduced.

For dilution of the liquid 20 per cent. glycerine should be employed. The dilutions are preferably made in the following manner.

1. With a 1 c.c. pipette, calibrated to $\frac{1}{10}$, 0·3 c.c. is withdrawn from the bottle, and mixed with 2·7 c.c. of the 20 per cent. glycerine solution, making in all 3 c.c.

This 10 per cent. dilution contains three milligrams of solid substance.

2. From this 10 per cent. dilution 0·1 c.c. is taken and made up to 10 c.c. with glycerine solution. Thus a 1 per mille dilution of the original fluid is obtained. Two divisions or $\frac{2}{10}$ c.c. of a Koch or Pravaz syringe of this dilution therefore contains $\frac{1}{500}$ of a milligram of solid substance.

Instruments and pipettes must, before use, be sterilised with absolute alcohol and ether and then rinsed out with sterilised glycerine solution, in order to remove every trace of alcohol and ether.

N.B. The 20 per cent. glycerine solution is prepared by mixing 20 c.c. of pure glycerine and 80 c.c. of distilled water and boiling for 15 minutes and then cooling thoroughly before use.

Dilutions which present a turbid appearance, or show a deposit which does not dissolve upon shaking, must not be employed. Generally the dilutions keep well for a fortnight in a cool and dark place.

The injections are made subcutaneously about every second day, the dose being raised so gradually that a rise in temperature of more than half a degree is as far as possible avoided. Any febrile symptom caused by the injection must have entirely disappeared before a fresh injection is made. With doses of 5 milligrams of solid substance and upwards it is not advisable to make more than two injections within the week, and with still larger doses not more than one. The individuality of the patient has generally to be taken into account.

As a rule the dose is increased to 20 milligrams of solid substance, and if no reaction follows the injection of this dose, the treatment is discontinued or only repeated at long intervals.

For injection, such parts of the body should be selected at which large folds of skin may be raised. The local reaction that not unfrequently appears in the locality of the injection generally disappears within twenty-four hours, and must be taken into account in increasing the dose.

Effects and Value of Tuberculin Treatment

In all probability, cases of tuberculosis of glands, bones, and joints are not much benefitted by tuberculin treatment.

In lupus a wonderful improvement is manifested under the treatment, both with the old and with the new tuberculin. Cases treated by the old tuberculin (under the care of Mr. Watson Cheyne, which were watched throughout by the writer when house surgeon at King's College Hospital) appeared to be cured, though in many instances the disease recurred when the treatment was discontinued. How far such relapses might be prevented by the use of the remedy for a long period of time is a question that is worthy of study. In some instances combined surgical and tuberculin treatment might be employed with advantage.

It might also be of value to try the effect of combined tuberculin and light or X-ray treatment; the tediousness of the last two might thereby be lessened.

In visceral tuberculosis, especially phthisis, treatment by both the old and the new tuberculins has, until lately, fallen into discredit. Latterly, however, it has been revived and sanatorium treatment has been combined with the administration of tuberculin. Both with the old and with the new tuberculin the treatment must not be begun too late; it is useless when the disease is advanced and a fatal termination imminent. In mixed infections also little benefit is to be expected; this is generally the case in patients whose temperature rises above 38° C. As regards the new tuberculin, Koch states that in lupus the cutaneous lesion improves, although local reaction is slight. In phthisis the early injections are followed by a slight increase in the crepitant râles, but after a few injections the râles disappear and cough and expectoration diminish and ultimately cease. No alarming symptoms or untoward results occur, and the temperature of patients who show a daily rise becomes normal throughout. In a number of favourable cases all manifested considerable improvement, an improvement so marked that for the time being, at any rate, it might be termed a cure.

Wilkinson[1] gives a good survey of the treatment of pulmonary tuberculosis with tuberculin. He says 'I have used T R chiefly, but I have also used the old tuberculin alone or alternately with T R. It is impossible to give an impression of the results of treatment by mere groups and tables. Personal experience has a value of its own that cannot be disregarded, and my personal experience in a large number of cases tells me that tuberculin, properly used, has a very high value in the treatment of pulmonary tuberculosis. I have carefully watched the effects week by week for nearly five years, and I assert that while the degree of improvement varies, and the duration of the improvement varies even more, gradual improvement is the almost invariable rule. I have treated nearly 70 cases and I do not remember a single instance in which there was no improvement, provided, of course, there was no mixed infection. Such uniform improvement under the tuberculin treatment must be due to the tuberculin, for tuberculin was the only uniform remedy. By improvement I mean that the cough grows less and less and ceases, the sputum diminishes in quantity, becomes, if yellow at first, opaque, white, and then clear and frothy, and finally ceases. In all cases in stage 1 (*i.e.* commencing consolidation) the tubercle bacilli, if found before treatment began, disappeared from the sputum during treatment, and later there was no sputum. In many cases in stage 2 (*i.e.* consolidation with commencing breaking down) tubercle bacilli disappeared from the sputum for months or years, or altogether. I never stopped treatment on account of hæmoptysis, and though there were many cases of severe hæmorrhage at one time or other, hæmoptysis was extremely rare after treatment. The effect of tuberculin treatment in cases of hæmoptysis is worthy of close attention, because if tuberculin had the effect, once attributed to it, of causing rapid and extensive softening, it should surely increase the tendency to hæmorrhage. My experience is that it does the very reverse, and that in a striking manner. The rapid and extensive softening, if it has been observed, has been due to mixed infection, not to tuberculin, mixed infection may certainly cause hæmorrhage. Tuberculin treatment, then, is specially indicated in cases of hæmoptysis, checking the hæmorrhage and preventing its recurrence.

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[1] *Brit. Med. Jour.* 1902, i. p. 1389.

'Like many other observers, I have never seen any evidence in favour of the idea that tuberculin may mobilise a dangerous enemy peacefully sleeping. In no instance did tuberculous meningitis supervene. If, then, tuberculin does not favour hæmorrhage, but rather checks it; if tuberculin checks, and may completely arrest, the cough; if tuberculin does not mobilise tubercle bacilli; if tuberculin diminishes expectoration instead of increasing it, and causes tubercle bacilli to disappear from the sputum, indicating most surely that, so far from favouring disintegration, it favours a healing process; if, under or after tuberculin treatment, patients invariably gain in weight and strength, beyond a doubt all the dangers that were held to be associated with tuberculin treatment vanish into mist. They were ghosts and shadows, unreal and unsubstantial, which no rational being need fear. I can vouch for improvement in all cases, provided there is no mixed infection. In many cases other physicians have witnessed the effects of the improvement and admitted the improvement. I have not yet seen a case of pure tuberculosis that did not improve greatly.'

Diagnosis of Tuberculosis in Man by means of Tuberculin

For this purpose the *old* tuberculin is used. Since the tuberculin, even in comparatively large doses, produces no effect in a non-tubercular individual, while in a tubercular one minute doses cause a rise of temperature, this reaction may be made use of for diagnostic purposes in suspected cases of obscure tuberculosis. The concentrated fluid is diluted with 0·5 per cent. aqueous carbolic, and injected in precisely the same manner as for treatment (see p. 204).

The following doses refer to the concentrated (i.e. *undiluted*) *fluid.*—The usual dosage is to commence with 0·001 c.c.; if this produces no result, 0·002 c.c. may be given after an interval of two days; if this again produces no reaction, 0·005 c.c. may be given after a further interval of two days; if no reaction is obtained with this dose, the condition may be considered to be non-tubercular. In certain instances, if these three injections produce no reaction, a fourth of 0·01 c.c. may be given. The reaction consists of a rise of temperature above the normal of 2° F. or more, with general malaise, and swelling, redness, desquamation, and irritability of local tubercular lesions, such as glands, and ulcers. The temperature should be taken every two hours. A rise of temperature of no more than 1°F. if it does not occur before or after, and coincides with, the injection, may be considered sufficient, especially if it happens again on giving a slightly larger dose.

Diagnosis of Tuberculosis in Cattle by means of Tuberculin

For this purpose the *old* tuberculin is employed in doses corresponding to about 0·1–0·2 c.c. of the concentrated fluid. The reaction consists in a rise of temperature above the normal, and the mode of applying the test is indicated in the following directions, issued by the Royal Veterinary College, London.

DIRECTIONS FOR USING TUBERCULIN

1. While under the tuberculin test cattle ought to be kept in the house, fed on their usual food, and protected from draughts. They ought not to be allowed to drink large quantities of cold water between the 6th and 15th hours after injection. It is well to take their temperature at least once on the day preceding the test.

2. The dose of tuberculin for a medium-sized cow is 3 cubic centimetres, or 50 minims, and it may be varied above or below that according to the size of the animal. Large bulls ought to receive 4 c.c.

3. It ought to be injected under the skin with a clean hypodermic syringe. The most convenient points are in front of the shoulder, or on the chest wall behind the point of the elbow. The best form of syringe is one with an asbestos piston, as the whole instrument

may be sterilised by boiling it in water for five minutes before use.

4. The tuberculin must be injected into the subcutaneous connective tissue, and care must be taken that the whole dose is introduced.

5. The temperature must be taken at the time of injection, and at the 9th, 12th, and 15th hours afterwards.

6. Animals in which the temperature during the fifteen hours following the injection rises *gradually* to 104° or more may be classed as *tuberculous*, and those in which it remains under 103° *as not tuberculous*. When the maximum temperature attained is under 104° but over 103°, the case must be considered doubtful, and the animal may be re-tested after a month.

7. The test is not reliable in the case of animals in the last stage of the disease, or in those in which the temperature is over 103° before injection.

8. The tuberculin should be kept in a cool place, and protected from light. Should it become turbid or cloudy it must not be used.

9. The tuberculin test does not render the milk in any way injurious.

The proper dose, as indicated in the directions issued with each brand, should of course be given.

Employed in this manner, and with the limitations indicated above, tuberculin is acknowledged by all veterinary authorities to be the most certain test for tuberculosis in cattle, especially when incipient and showing no manifest clinical symptoms. It is quite harmless, and does not affect the milk.

By repeated injections (three or four) of tuberculin, an immunity is induced, so that, if so treated, a tuberculous beast may not react when subsequently injected, and this method has been employed to evade the enactments against the admission of tuberculous cattle into certain countries.

Treatment of Pulmonary Tuberculosis with Filtered Tubercle Cultures

Denys details a method he has employed for the treatment of pulmonary tuberculosis somewhat similar to the old tuberculin treatment (*Bull. de l'Acad. Roy. de Méd. de Belg.* t. xvi. No. 3, 1902, p. 153). Apparently cultures of the tubercle bacillus (? virulent) in glycerine broth are filtered through a porcelain filter and the filtrate is employed for injection. The culture is not heated. In cases without fever, treatment is commenced with small doses, $\frac{1}{10}-\frac{5}{10}$ milligram. This produces a febrile reaction, and the same dose is administered, until tolerance is established, and if this does not occur after two or three doses, the dose is diminished to one half or one quarter. The dose is increased as follows :

up to 1 milligram by $\frac{1}{10}$ milligram each time; up to 1 centigram by 1 milligram; up to 1 decigram by $\frac{1}{2}$ centigram; up to 1 gram by $\frac{1}{2}$ decigram. Should febrile attacks occur independently of the injections, it is well to reduce the dose.

In febrile cases, after rest in bed, &c. for a fortnight, a dose of $\frac{1}{100}$ milligram is given, and injections are administered every second day, slowly and gradually increasing the dose.

By this method, tested on some fifty patients, Denys claims that there is considerable improvement in the physical signs, diminution and generally cessation of the cough and expectoration, and disappearance of the bacilli, increase of the appetite and gain in weight, cessation of fever and of night sweats when present.

Mallein

Mallein is a fluid employed for the diagnosis of glanders and is an analogous preparation to the old tuberculin (see p. 199), only substituting the glanders bacillus (*B. mallei*) for the tubercle bacillus. The shallow flasks of veal broth are inoculated with a *virulent* culture of the glanders bacillus, and grown for three to four weeks; the culture is then autoclaved, concentrated to one-tenth of its volume and filtered. The mode of preparation is practically precisely the same as for the old tuberculin.

Mallein is rarely if at all employed on human patients, and then only for diagnostic purposes. It possesses little, if any, curative properties. Its chief use is for the diagnosis of glanders in the horse or other equines, and for this purpose it is admittedly the most certain test, especially in the early stages before the clinical signs are obvious.

The reaction consists in a rise in temperature above the normal, and in the formation of a local swelling at the seat of inoculation, as indicated in the following directions issued by the Royal Veterinary College, London.

DIRECTIONS FOR USING MALLEIN

1. While under the mallein test, horses ought to be left at rest in the stable and protected from draughts. The rectal temperature ought to be taken once or twice on the day before the test is applied.

2. The dose of mallein for a horse is 1 cubic centimetre, or 18 minims. It ought to be injected about the middle of the side of the neck, with a clean hypodermic syringe. The best form of syringe is one with an asbestos piston, as the whole instrument may then be sterilised by boiling it in water for five minutes before use.

3. The mallein must be injected into the subcutaneous connective tissue, and care must be taken that the whole dose is actually introduced.

4. The temperature must be taken at the time of injection, and at the 9th, 12th, and 15th hours afterwards.

5. Provided the temperature was normal (under 101° F.) before the injection, it will rise 2° or more (103°–105°) during the next fifteen hours if the horse is glandered, but it will remain practically unaffected (under 102°) if the horse is not glandered.

6. Attention must also be paid to the swelling that forms at the seat of injection. When the horse is glandered this goes on increasing in size during the second twenty-four hours after the injection, and it seldom declines before the third or fourth day. The maximum diameter of this swelling in glandered horses varies from 5 to 10 inches.

7. In horses that are not glandered the local swelling attains its maximum size during the first fifteen hours, and by the twenty-fourth hour it has almost entirely disappeared. Its maximum diameter is usually about 3 or 4 inches.

8. When the temperature gradually rises from the normal to 104° during the first fifteen hours, and a large slowly disappearing swelling forms at the seat of injection, the horse may confidently be declared glandered.

9. If, with a normal temperature at the time of injection, a horse displays only the temperature reaction, or only the local reaction, the case must be considered doubtful, and the test repeated after the lapse of a week.

10. When the temperature is 102° or more at the time of injection the temperature reaction is unreliable, but in such a case the diagnosis may be based on the characters of the local swelling.

11. The mallein should be kept in a cool place, and protected from light. Should it lose its transparency, or become cloudy, it must not be used.

The proper dose, as indicated in the directions issued with each brand, should of course be given.

CHAPTER VIII.

METHODS OF PRODUCING ACTIVE IMMUNITY—VACCINE LYMPH—ANTI-RABIC INOCULATION—ANTI-CHOLERA VACCINE—ANTI-TYPHOID VACCINE—ANTI-PLAGUE VACCINE—COLEY'S FLUID—CANCROIN—THERAPEUTIC USE OF YEAST

As mentioned before, owing to the failure to obtain anti-sera for a number of diseases, especially cholera, typhoid fever, and septic conditions due to staphylococci, there has recently been a return to the Pasteurian system of producing an active immunity by means of vaccines consisting of sterilised bacterial cultures.

The use of living cultivations the virulence of which has been artificially diminished in some way—*e.g.* by continuous culture *in vitro*, or passage through other animals, by culture at a high temperature, in the presence of traces of antiseptics, &c.—is, for man, confined to vaccination with vaccine lymph (anti-variola vaccine), and to the Pasteurian system of anti-rabic inoculation. For the domestic animals, however, this method is still largely employed, notably in anthrax, quarter-evil, and rinderpest.

Both these methods of active immunisation are used almost exclusively as a means of *prevention* and not for *cure*. In certain instances, however, when the infection is localised or is a very chronic one, it might be practicable to use them so as to rapidly increase the bactericidal power of the blood, and thus to bring the infection to an end. Wright[1] has suggested this mode of treatment in furunculosis, sycosis, acne, &c., which seem to be generally due to a staphylococcic infection, and might be treated with a vaccine prepared with the *staphylococcus pyogenes aureus*,[2] and in cholelithiasis, cystitis, pyelitis, &c., due to infection with the colon bacillus, with a *B. coli* vaccine.

Wright[3] has pointed out that the first effect of the injection of a bacterial vaccine (also of tuberculin) is a *diminution* of the resisting power of the individual as measured by the phagocytic power of the blood; this Wright terms the 'negative phase,' which is rapidly succeeded by an increased resistance or 'positive phase.' Should a second injection be given during the negative phase and before the positive phase has developed, a still more marked diminution of the resisting power will be produced, a result the reverse of that desired. In order to obtain the maximum beneficial effect, the inoculations should be so arranged that succeeding inoculations are given at the height of the positive phase of the previous one. This may be done by systematically testing the phagocytic power of the blood by Leishman's method,[1] or, in the case of tuberculin, by estimating the agglutinating power of the blood.

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[1] *Brit. Med. Journ.*, 1903, i. p. 1069 (general considerations and suggestion in this direction).

[2] Cases treated by Wright, *Lancet*, 1902, i., March 26.

[3] *Lancet*, 1903, i. p. 1299.

Vaccine Lymph

At the present time, arm-to-arm vaccination, undoubtedly the most efficient form, has almost entirely been given up, owing to the risk of conveying such diseases as syphilis. For it, calf lymph has been substituted, and in order to destroy pyogenic or other organisms, which are not essential, and may cause unpleasant or dangerous effects, the lymph is treated with glycerine, which destroys these micro-organisms, but leaves uninjured the true vaccinating agent. This is the well-known glycerinated lymph, which is almost exclusively employed now-a-days.

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[1] *Brit. Med. Jour.*, 1902 i. p. 73.

The mode of preparation of glycerinated lymph, as carried out in the laboratories of the Local Government Board, is as follows :[1]

'Calves of suitable age (three to six months), breed, and condition, are kept in quarantine for a week to see that they are healthy. If so, when required for vaccinating purposes, the calf is strapped to a large tilting table, and the lower part of the abdomen, extending as far forward as the umbilicus and backwards into the flanks, is carefully shaved. This shaved area is first washed with a 5 per cent. solution of carbolic acid or lysol, then well syringed with tap-water, and finally cleansed with sterilised water. The moisture from such washing is removed from this shaved area, and from the adjacent skin, by means of sterilised gauze sponges.

'The calf is then vaccinated with glycerinated calf lymph, introduced into the skin in numerous parallel linear incisions by a sharp scalpel, previously sterilised, which is dipped from time to time in the vaccinating fluid. The incisions are designed to penetrate the epidermis and to open up the rete Malpighii, if possible without drawing blood ; and as they are made, additional glycerinated lymph is run in along the whole length by the aid of a sterilised blunt instrument, such as an ivory or bone spatula. The inoculation of the incisions is effected immediately they are made, otherwise the lips of the wound are apt to swell and close the opening. After vaccination, the calf is removed from the table, and is then so stalled in a stable as to prevent any injury to the vaccinated surface. The temperature of this stable is not allowed to fall below 60° Fahr.

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[1] Report by Dr. Blaxall, *Twenty-eighth Ann. Rep. Loc. Gov. Board*, *Supplement containing Rep. of Med. Officer for* 1898–99, p. 35.

'*Collection of the Vaccine material.*—After five days (120 hours) the calf is again placed on the table, and the vaccinated surface is thoroughly washed with soap and warm water, gently rubbed over it by the clean hands of the operator. It is again washed with tap-water and finally cleansed with sterilised water. Next, any crusts that may have formed upon the vesicular lines and any epidermal débris are removed by the careful use of a sterilised india-rubber pad. Superfluous moisture is absorbed by sterilised gauze sponges. At this stage the site of each incision should present a line of continuous vesiculation.

'The skin having been put firmly on the stretch, the vesicles and their contents are collected with a sterilised Volkmann's spoon, each line being treated in turn and scraped once only, care being taken that the edge of the spoon does not touch the neighbouring lines of vesicles. In this way the vesicular pulp is removed without admixture of blood. The pulp obtained by the above procedure is received into a previously sterilised stoppered bottle of known weight.

'The abraded surface of the calf is gently washed with warm water, and dusted over with starch powder or boracic acid powder. The calves after use should be slaughtered and the carcases carefully examined. If any carcase show disease, the lymph from that animal should be discarded.

'*Glycerination of the Vaccine material.*—The bottle containing the lymph pulp from each calf is then again weighed so that the exact weight of the material is ascertained. The pulp is next transferred to a triturating machine; that employed being either one invented by Dr. Chalybäus, of Dresden, or a modified form of it. All the parts of the machine which come in contact with the lymph pulp are previously sterilised by prolonged steaming. The vaccine material, just as it is derived from the calf, is then passed through the machine, which is worked by an electric motor. When the pulp has been triturated in this way, the amount of subdivision it has undergone can be ascertained by suspending a loopful of the ground-up material in a watch glass containing distilled water. If the trituration has been effectual, such suspension should show only the minutest particles of pulp: causing the water to appear merely cloudy. The pulp is then passed through the machine a second time, together with six times its weight of a sterilised mixture of 50 per cent. pure glycerine in distilled water. The resulting mixture is then once more passed through the machine; thus producing a fine and intimate emulsion. At this stage a loopful of the emulsion is withdrawn with a sterilised platinum needle, and agar-agar plates are established, in order to estimate both the number and the quality of the organisms present in the lymph.

'*Storage of Emulsion.*—The emulsion is next received into conical glass receptacles, previously sterilised. By means of a stopcock at the point of the cone, the glycerinated lymph is run into small sterilised test-tubes capable of holding 4 to 10 c.c. Each tube is filled as completely as possible, so that very little air remains in contact with the emulsion. It is plugged with a sterilised cork, is sealed with melted paraffin, which has been rendered aseptic with carbolic acid, and is then placed in a dark cool cupboard or ice-chest. Week by week, agar-agar plates are established from the emulsion, with the result that the number of colonies is shown to diminish successively in the several plate cultures. At the end of a month the plates rarely show colonies of any sort. When the stage is reached at which agar plates show no growth after inoculation with the emulsion, samples of the lymph are drawn up into capillary tubes, and may be tested on children. The results of these vaccinations are recorded a week later, and from the number and size of the vesicles obtained, an estimate is made as to the potency of the lymph. If satisfactory, the lymph is then filled into the fine-bore vaccine tubes, which are sealed in a flame and are then ready for distribution.' The tubes are usually filled by a machine which drives the lymph into them by means of compressed air.

The use of unripe lymph—*i.e.* lymph with glycerine but not kept sufficiently long for the glycerine to destroy the contaminating microbes—is followed by excessive fever, inflammation, pus production, and other symptoms of septic infection. The use of stale lymph—that is, lymph that has been kept so long that the glycerine is beginning to destroy the vitality of the vaccine organism—is a less serious evil. Copeman has stated that the glycerinated lymph may be kept for considerable periods of time without deterioration—*e.g.* for eight months or even longer (‘Milroy Lectures,’ 1897)—but the Chicago Health Department does not agree with this view. Their experience is that in the glycerinated lymph, stored under proper conditions, the extraneous pathogenic organisms are destroyed in from fifty to sixty days, and that at the end of this period the lymph continues active for another period of forty or fifty days, after which it deteriorates.

The amount of glycerine added to the lymph varies at different establishments.[1] At Berlin the proportions are :—

Vesicle pulp . .	1 part.
Glycerine . . .	7 parts.
Boiled water . .	7 ,,

At Cologne,

Vesicle pulp . .	1 part.
Glycerine . . .	10 parts.
Water . . .	5 ,,

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[1] *Rep. to the Loc. Gov. Board on the Prep. and Storage of Glyc. Calf Vaccine Lymph*, 1897.

At Geneva,

Vesicle pulp	1 part.
Glycerine	2 parts.
Water	1 part.

Green[1] has found that the vesicle pulp, finely ground, and mixed with five or six times its volume of saturated chloroform water, soon becomes freed from extraneous organisms. His most recent method is to pass air charged with chloroform vapour through the emulsified vesicle pulp. This kills the extraneous micro-organisms in a few hours.

No unbiassed person can doubt the protective power of vaccination against variola. In many instances, however, vaccination is performed in a perfunctory manner, even by medical men who should know better. At least three, preferably four, places should be inoculated, and vaccination should be repeated at intervals of a few years (7–10) until late adult life, especially if an epidemic of variola is in progress.

It can now hardly be doubted that vaccinia is modified variola, and this would explain the rationale of the process. In a few instances variola has been inoculated upon the calf and after three or four passages becomes indistinguishable from true vaccinia. It is very difficult, however, to inoculate the calf successfully from a case of variola, but Copeman has recently shown that *inoculated* small-pox can be much more readily inoculated upon the calf, and he suggests therefore that spontaneous vaccinia has developed from *inoculated* small-pox, since small-pox inoculation was practised at the time Jenner made his discovery.

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[1] *Thirtieth Ann. Rep. Loc. Gov. Board*, *Rep. Med. Off.* for 1900–01, p. 639, and Royal Society, London, April 30, 1903.

Anti-rabic Inoculation (for Hydrophobia or Rabies)

Hydrophobia or rabies, the ætiological microorganism of which is not known with certainty, is caused by the bites of rabid animals. The infective agent, whatever it be, resides in the central nervous system, in the saliva, the lachrymal glands and suprarenal capsules, but the other tissues and fluids of the body are non-infective.

Hydrophobia attacking man is invariably contracted through the bite of an animal affected with the disease. It is most frequent in the dog, but the cat, wolf, and deer are also subject to it, and other animals can be infected by inoculation. In the lower animals the disease is termed rabies, and takes two forms, either the raging one or the paralytic. The latter is not met with in man, unless certain rare forms of acute ascending paralysis (*e.g.* Landry's) be manifestations of it. In the dog either may occur, but in rodents the paralytic form is almost always the one it assumes. In man the incubation period is very variable; it is never less than about twenty days, and may possibly be as long as two years, or even more; the average seems to be about ten weeks. In the rabbit after inoculation from the dog, the incubation period is about two to three weeks.

Pasteur showed that the virus could be attenuated by desiccating the infective nerve matter, and in this way was able to prepare a vaccine which would protect animals from otherwise fatal doses of the virus. Advancing a step further, he used his vaccines to treat individuals who had been bitten by rabid animals, but in whom the symptoms had not yet developed, and so inaugurated the present system of anti-rabic inoculation as carried out at the Pasteur and other Institutes.

To prepare the anti-rabic vaccines a rabbit is inoculated subdurally with an emulsion prepared from the medulla of a rabid dog. When the animal dies a second rabbit is similarly inoculated from the first, and the passage through rabbits is continued until a ‘fixed virus’ is obtained, with which the first symptoms appear on the seventh or eighth day, and which kills with certainty in about ten days. This having being attained, two or three rabbits are inoculated subdurally every day, so that there is a daily supply of animals dead of the disease. The spinal cord is removed with aseptic precautions, and cut into convenient segments, which are suspended in bell-jars containing a layer of caustic potash at the bottom; this serves to desiccate them. The jars are dated and preserved in glass cases in a dark room, and kept at a constant temperature of about 23° C. In Paris the vaccine fluids are prepared by triturating portions of the dried cords in sterile broth, so as to form an emulsion—1 centimetre of cord in 5 c.c. of sterile broth, of which 1 c.c. (*i.e.* 2 mm. of cord) forms a single dose. At the commencement of treatment the cords which have been dried for fourteen days are used, at the end of treatment those which have been dried for only three days; the latter are much more virulent, and would communicate the disease but for the previous treatment. The rabbits employed should all be of the same weight ($2\frac{1}{2}$ kilograms at Paris); if the rabbits are small, a slightly shorter period of desiccation of the cords would be necessary. The treatment varies in duration according to the severity of the case, which is gauged by the number and situation of the bites and by the species of animal. Bites on exposed parts are regarded as much more serious than those through clothing, and on the face, where efficient treatment is difficult, than on the hands, and wolf bites than dog bites.

The doses are injected subcutaneously in the flank, and do not produce much constitutional disturbance. At first there is a feeling of lassitude and considerable muscular tenderness at the seat of inoculation, which later on passes off. At Lille, where there are only a few cases under treatment at a time, the cords after drying for the requisite period are placed in pure sterile glycerine. In this they retain their virulence unimpaired for about a month. This method does away with the necessity for the daily inoculation of rabbits, a rabbit being inoculated occasionally as required. The system of dosage employed at the various anti-rabic stations differs somewhat; the following is that employed at Lille, 2 mm. of cord being emulsified in 5 c.c. of sterile broth or physiological salt solution :

Ordinary Treatment

Day of Treatment	Days of Desiccation of Cord
1 (two injections)	14 and 13
2 ,,	12 and 11
3 ,,	10 and 9
4 ,,	8 and 7
5	6
6	5
7	4
8	3
9 (two injections)	9 and 8
10 ,,	7 and 6
11	5
12	4
13	3
14 (two injections)	9 and 8
15 ,,	7 and 6
16	5
17	4
18	3
For Severe Bites, in Addition	
19 (two injections)	7 and 6
20	5 and 4
21	3

At Buda-Pest a dilution method has been employed ; instead of drying the cords, an emulsion is made with the fresh cord, and this emulsion is considerably diluted for the earlier doses, dilutions of 1 in 10,000 to 1 in 6,000 corresponding to cords dried for from fourteen to eight days.

Undoubtedly the Pasteur inoculations will protect animals from rabies, the duration of immunity after vaccination in the dog being at least three years. In man the efficacy of the treatment can only be judged by statistics. The mortality after bites by supposed rabid animals is variously stated, the most favourable being about 16 per cent. (Leblanc). During 1901, 1,321 persons were treated at the Pasteur Institute, of whom 5 died from hydrophobia more than fourteen days after the termination of the treatment, a mortality of only 0·38 per cent. The previous year (1900) the mortality among 1,413 persons treated was only 0·28 per cent. In 2,730 cases treated in which the animal which inflicted the bites was proved to be rabid by inoculation experiments, only 19 deaths occurred, a mortality of 0·7 per cent.

The failure of the treatment may be due to two causes : (1) delay in its commencement, and (2) a short incubation period. The principle of the treatment probably depends upon the long incubation period of the disease, owing to which it is possible to forestall the disease and to immunise the body by the inoculations before its onset. If, unfortunately, the infective material should be very virulent, and the incubation period thereby reduced to the lower limit, it may be impossible to do this before the onset of the disease, and the same is the case if the commencement of the treatment be delayed. Pasteur's system of inoculation is useless when the disease has declared itself.

Anti-cholera Vaccine

The vaccines employed in the anti-cholera vaccination are two in number, a first or weak, and a second or strong. The following is Haffkine's method[1] :—

The first vaccine is prepared from attenuated cultures of the cholera spirillum. The ordinary laboratory cultures are usually considerably attenuated, but to be sure that they are sufficiently so they are grown for several generations on surface agar at 38° C. in tubes through which a current of moist air is continuously passed. Such a culture causes only a local œdema instead of necrosis when injected into the subcutaneous tissue of a guinea-pig.

The second, or strong vaccine, is prepared from cholera cultures, the virulence of which has been artificially increased by growing in the peritoneal cavity of guinea-pigs. This is done by first of all preparing standard cultures from any ordinary culture of the cholera vibrio. Test-tubes measuring 15 cm. in length are employed; of the 15 cm., 10 cm. are occupied by the sloping surface of ordinary nutrient agar. The whole surface of the nutrient medium is inoculated and the inoculated tubes are incubated at 35° C. for twenty-four hours. The whole growth from the surface of the agar is then scraped off with a sterilised platinum needle of stout wire and made into an emulsion with about 3 c.c. of sterile broth. A guinea-pig (300–400 grams) is etherised, a small patch of hair on the abdomen cut short, and a spot cauterised with a hot iron to sterilise it. The emulsion of cholera bacilli is then drawn up into a sterile syringe or glass pipette and injected into the abdominal cavity through the cauterised area. Two guinea-pigs should be injected at the same time, using for each one a standard cholera culture. The guinea-pigs so treated will die within twenty-four hours.

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[1] *Brit. Med. Jour.*, 1893, i. p. 227 (Wright and Bruce).

The animals are then pinned out and the peritoneal cavity is opened with strict aseptic precautions; with a sterile forceps the intestines are thrown upwards and to the right, and with a sterile glass pipette the peritoneal fluid is sucked up from the iliac fossæ. The whole of the peritoneal fluid from one guinea-pig is introduced into a sterile test-tube which is well plugged with cotton-wool and placed in the oblique position (for aëration) in the incubator at 35° C. for about ten hours. This is to allow of the proliferation of the cholera bacilli. After this treatment the fluid is similarly injected into a second guinea-pig, the size of which, however, has to be taken into account. If the peritoneal fluid in the first guinea-pig be abundant it will contain comparatively few cholera bacilli, and a smaller animal should be chosen, but if it be scanty the comma bacilli will be numerous, and a larger animal may be used. After twenty to thirty passages through guinea-pigs the virus will have attained its maximum virulence, which is known by the fact that further passages do not shorten the period that elapses between inoculation and death.

Throughout all the manipulations the greatest care must be taken to prevent contamination, and the cultures, &c. should be controlled by subculturing and microscopical examination. The 'exalted' cholera cultures do not retain their maximum virulence for longer than ten days, and have again to be passed through guinea-pigs (three or four).

In order to prepare the vaccines a 'standard' agar tube is inoculated over its whole surface and incubated at 35° C. for twenty-four hours. Three or four cubic centimetres of sterile broth are introduced into the tube and an emulsion is made with the whole of the growth. The emulsion is measured by drawing it up into a sterile syringe, the contents of which are then introduced into another sterile glass and made up to a volume of 8 c.c. by the addition of more sterile broth. One cubic centimetre of this emulsion constitutes the dose for vaccination. Carbolised vaccines may be prepared by using a ½ per cent. solution of carbolic acid (sterilised by boiling) for making the emulsions and diluting them to 6 c.c., and not 8 c.c., as in the uncarbolised. The carbolised vaccines may be preserved for some time in sealed tubes.

The dose of vaccine (1 c.c.) is injected hypodermically into the flank, the second or strong vaccine being injected three to five days after the first or weak one.

The statistics of the value of anti-cholera inoculation are very favourable. In a certain district in Calcutta, Simpson had under observation for a period of two years 8,000 individuals who had been inoculated; these lived with their uninoculated relatives and neighbours in the same huts. During 738 days, cholera occurred among the uninoculated on 78 days, among the inoculated on only 10 days. From the 4th to the 216th day after inoculation, *one* case only of cholera occurred among the inoculated, and from the 5th to the 420th day after inoculation the number of deaths among the inoculated was 22·62 times smaller than among the uninoculated. The case-mortality, however, does not seem to be influenced by the inoculations.[1]

Anti-typhoid Vaccine[2]

A virulent typhoid bacillus (the virulence being kept up by intraperitoneal passage through guinea-pigs) is grown in peptone beef broth in flasks at 37° C. for from fourteen to twenty-one days. The flasks are then so heated that their contents attain, and remain at for a few minutes, a temperature of 60° C. To obtain uniform toxicity the contents of several flasks should be mixed, and to safeguard the vaccine from contamination one-tenth of its volume of 5 per cent. lysol or carbolic acid is added. Various ingenious devices have been adopted by Wright and Leishman (*loc. cit.*) to prevent contamination and for standardisation.

The dosage and strength of the anti-typhoid vaccines.—The strength of a typhoid vaccine depends upon the number of bacilli it contains and their virulence. It is standardised in two ways, by ascertaining the degree of opacity and by determining the toxicity. The former is accomplished by measuring the thickness of fluid which will just obscure a test object (strips of black paper pasted on a slide) under standard conditions of illumination and magnification. As regards toxicity, this is ascertained by subcutaneous inoculation of guinea-pigs weighing 250–300 grams. Eight animals are employed, and each couple receives 0·5, 0·75, 1, and 1·5 c.c. respectively. The maximum toxicity observed corresponds to about 0·5 c.c. per 100 grams of body weight as the minimal lethal dose, the minimum toxicity to 2 c.c. per 100 grams. Of the former or stronger 0·5 c.c., and of the weaker 1·5 c.c., is the dose for man, which is injected subcutaneously in the flank.

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[1] Haffkine, *Brit. Med. Jour.*, 1899, ii. p. 12.

[2] See Wright and Semple *Brit. Med. Journ.*, 1897, i. p. 256, and Wright and Leishman, *ibid.* 1900, i. p. 122.

Clinical symptoms which supervene upon the inoculation of the anti-typhoid vaccines.—The symptoms are comparatively slight when small doses are used, such as tenderness at the seat of inoculation, and two or three hours after inoculation a chilly feeling, slight rise of temperature, and restlessness at night, but these symptoms pass away in about twenty-four hours. With larger doses all the symptoms are severe, and are described as commencing two or three hours after injection, with tenderness, which gradually increases in severity and extends upwards into the armpits and downwards into the groin, a patch of congestion two or three inches in diameter develops round the site of inoculation, and red lines of inflamed lymphatics can be traced extending into the armpits. These symptoms gradually subside in about forty-eight hours.

The constitutional symptoms are marked by some degree of faintness and collapse, in some cases accompanied by nausea and vomiting, which commence about three or four hours after injection, entire loss of appetite, disturbed sleep, and high temperature, all of which pass off in a few hours. The blood and serum of individuals vaccinated in this way give the agglutination reaction in a marked manner.

To obtain more complete protection a second inoculation of one-and-a-half to twice the original dose should be given after an interval of ten to fourteen days. There is practically no risk, and the immunity conferred will probably last for some years.

As regards the sphere of application of the anti-typhoid vaccination, it might be expedient in the case of young soldiers and other individuals going abroad to infected districts, for those living in a district visited by an epidemic, or for those in attendance upon typhoid patients.

The value of anti-typhoid inoculation is still *sub judice* and can only be gauged by statistics. From an analysis of statistics dealing with some 15,000 inoculated individuals, mostly soldiers, in Great Britain, India, Egypt, Cyprus, and South Africa, Wright[1] deduces the following conclusions: (1) As regards the *incidence* of typhoid fever, there is a reduction of at least twofold, but it may be as great as 28-fold; (2) As regards *case-mortality* there is a reduction of one half; (3) as regards *death-rate* there is at least a twofold reduction, generally a fourfold one, among the inoculated.

Anti-plague Vaccine

An anti-plague vaccine was first prepared by Haffkine, is frequently termed the Haffkine prophylactic, and is the one most generally employed. The method of preparation is comparatively simple. A virulent plague bacillus is cultivated in flasks in a special broth; this consists of ordinary peptone beef broth to which a trace of butter is added. In Hindu countries, in which the cow is sacred, goat's flesh may be substituted for beef for making the broth. The butter melts at the temperature of incubation (35° C.) and forms little islets upon the surface of the medium which serve as nuclei from which growth starts. The flasks must be kept absolutely still, in which case copious flocculent pyramidal growths depend downwards into the medium forming the so-called stalactite growth of Haffkine. This is shaken down, allowed to reform, again shaken down, and the process is repeated several times. Haffkine gives the following details of the mode of preparation of his prophylactic.[1] Mutton is finely minced and infused in dilute hydrochloric acid. The exact proportions of the materials are as follows: 1½ kilos of mutton are infused in 3 litres of water plus 225 cubic centimetres of hydrochloric acid. As a rule, the material is kept in this infusion for two or three days in the cold. Afterwards it is subjected to a high temperature, 130° to 140° C., which corresponds to a pressure of about 2½ atmospheres. It is kept at that temperature for six hours. The fluid is then siphoned off, filtered, and the filtrate diluted with a sufficient quantity of water to bring the amount to 4½ litres. This solution is neutralised with 60 grams of caustic soda and again heated to a temperature equal to the previous one, for only half an hour, then filtered again, and whatever solid residue is produced by the neutralisation and second heating is again rejected, and only the liquid part employed. This liquid is called Warden's bouillon. For the cultivation of the plague prophylactic it is mixed with a small quantity of ghee or cocoanut oil, distributed into big flasks, sterilised, and inseminated with a minute quantity of the most virulent plague microbes which can be obtained, and the inoculated liquid is incubated. During the first two or three days scarcely any signs of change are observed, but then minute flakes appear underneath the suspended droplets of oil or ghee. These flakes in the course of from twelve to twenty-four hours grow down in the shape of icicles or stalactites. The liquid remains clear, except for a small quantity of powder-like residue, which very early in the process falls to the bottom of the flask. The stalactites in the course of two or three days fill the upper half, or sometimes even the whole volume, of the liquid. The least oscillation of the vessel is sufficient to detach the suspended 'icicles' from the drops of ghee or oil, and the whole growth in the course of a day or so falls to the bottom of the flask, while the liquid appears again perfectly limpid. After the first growth of stalactites has been brought down by shaking, a new crop of flakes appears underneath the droplets of oil or ghee. The same process as described above is repeated again, except that it may be a little slower in development. After another two or three days the flask is again shaken and the second crop brought down. This process is repeated ten or twelve times, the development taking from five to six weeks before it is perfectly accomplished, and the growth becoming slower and slower, until it stops entirely. The cultures are then sterilised by heating to 65° C. for one hour, and carbolic acid in the proportion of 0·5 per cent. is added. The dose employed is 2·5 c.c., which is injected into the flank. A second injection given a week after the first increases the immunity.

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[1] *Lancet*, 1902, ii. Sept. 6.

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[1] *Report of the Indian Plague Commission.*

Lustig and Galeotti[1] have also prepared an anti-plague vaccine by growing the plague bacillus upon agar for three days, scraping off the growth and treating with 1 per cent. caustic soda. The fluid is then filtered through paper and precipitated with dilute (0·1 per cent.) acetic or hydrochloric acid, or by saturation with ammonium sulphate. The precipitate is dissolved in a 0·5 per cent. solution of sodium carbonate, and filtered through a Chamberland filter. This is the vaccine fluid and the dose corresponds to 1 milligram of solid matter.

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[1] *Brit. Med. Journ.*, 1897, i. p. 1057, and 1900, i. p. 311.

Value of Anti-plague Inoculation

The value of the Haffkine anti-plague inoculation can only be estimated from statistics. The following table illustrates some of the results obtained :

Place	Number of Persons	Number of Cases of Plague	Number of Deaths from Plague	Mortality per cent.
Mora	Non-inoculated 581	26	24	—
"	Inoculated 419	7	0	—
Damaun	Non-inoculated 7,213	—	716	9·9
"	Inoculated 1,017	23	6	0·58
"	Non-inoculated 5,869	—	674	11·5
"	Inoculated 1,639	64	27	1 6
"	Non-inoculated 4,643	—	93	2·0
"	Inoculated 2,164	4	3	0·14
Kirkee	Non-inoculated 859	143	98	11·4
"	Inoculated 671	32	17	2·5
Khoja Community of Bombay	Non-inoculated 9,516	—	77	—
"	Inoculated 3,814	—	3	—
Hubli	Non-inoculated 17,786	—	2,348	—
"	Inoculated 24,631	—	338	—
				Case Mortality
Dharwar	Non-inoculated 16,848	1,100	889	80·3
"	Inoculated 4,321	129	54	41·8

The Bombay Plague, p. 35. Captain Condon. Bombay, 1900.

From this it is evident that both the incidence of plague, and the mortality among the inoculated who happen to contract the disease, are very much lessened by the inoculation.

Calmette has asserted as the outcome of his experiments upon animals, that a person inoculated with the prophylactic during the incubation period of the disease, would have the disease in so aggravated a form that it would almost certainly prove fatal. Bannerman,[1] however, finds that this is not the case; among persons inoculated with the prophylactic and who developed plague within ten days, the case-mortality was never more than 62·5 per cent., and averaged only 47·0 per cent., while among the uninoculated the case-mortality was 73·7 per cent. among the same population during the same period. Bannerman also believes that protection is secured within twenty-four hours of the inoculation, and that it lasts as long as eighteen months.

Calmette has suggested that in order to procure a *rapid* immunity, 10 c.c. of anti-plague serum may be injected, followed by the Haffkine prophylactic to obtain the prolonged immunity. Such a combined procedure might be expedient for those suddenly called upon to tend the sick. No statistics seem to have been published of the use of Lustig and Galeotti's vaccine.

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[1] *Centr. f. Bakt.* Abt. i. Bd. xxix. p. 873.

Coley's Fluid

This preparation consists of the toxins of the streptococcus of erysipelas and the *Bacillus prodigiosus.* It was devised by W. B. Coley, of New York, as a possible cure for inoperable malignant tumours. The treatment is based on the undoubted fact that malignant growths may decrease or even disappear completely after an attack of erysipelas. The fluid is prepared by growing the streptococcus, obtained from a fatal case of erysipelas, and rendered highly virulent by a succession of passages through rabbits, in bouillon for about ten days; the *B. prodigiosus* is now added and the two are allowed to grow together for another week or ten days. The culture is then heated to from 58° to 60° C. for one hour and a piece of thymol added to keep it.

The fluid is injected subcutaneously in the vicinity of the tumour. The earlier injections may be performed with the *filtered* toxin, which does not produce so much reaction as the unfiltered.

The dose to commence with should be 1 to 2 minims of the filtered, or ½ minim of the unfiltered, fluid. The dose is gradually increased each day until there is a temperature reaction of 103° to 104° F. The temperature is the chief guide in estimating the dose, and the frequency of injections depends upon the general condition of the patient and upon the rapidity of recovery from the depression of the preceding dose. The injection must not be repeated until the temperature has completely fallen. The injection is preferably made in the neighbourhood of the growth.

According to Coley's most recent statistics, he has treated in all 140 cases of sarcoma, of which 84 were of the round-celled type, 21 spindle-celled, 9 melanotic, 2 chondro-sarcomata. Of the round-celled, 40 were more or less improved but only 3 cured; of the 21 spindle-celled, 10 had disappeared completely and the remainder were much improved. The spindle-celled is therefore the variety most favourable for treatment. Of the cases cured, 16 had remained well for 3–8½ years; of these two had recurred at three and eight years respectively and both died.

The treatment does not appear to be nearly so successful in melanotic sarcoma or in carcinoma, only two or three cases of the latter having been permanently cured.

Coley himself advocates the treatment only in inoperable cases.

LITERATURE

Coley, *Amer. Journ. of Med. Sc.*, cxii., 1896, p. 251; *Johns Hopkins Hosp. Bull.*, vii. 1896, p. 157; *Ann. of Surgery*, xxv., 1897, p. 174; *ibid.* xxvi., 1897, p. 232; *Philad. Med. Journ.*, 1901, May 25, p. 1013.

Cancroin

Adamkiewicz has devised a mixture, termed by him 'cancroin,' which is stated by himself and others to be of service in the treatment of carcinoma. It was originally prepared by extracting carcinomatous tumours, but the active principle is stated to be identical with neurin, and an artificial substitute has been prepared, viz.:

	Parts
Neurin (25 per cent. solution) .	10
Ac. citric. to saturation . .	1·82
Ac. carbolic. to saturation .	1·25
Distilled water	27

The solution is diluted with an equal quantity of water, and one gram is injected.

See *Lancet*, 1902, i. p. 288 and p. 322; *Berl. klin. Wochenschr.*, 1902, No. 24, No. 28, and No. 36.

Yeast

Ordinary brewer's yeast (*Saccharomyces cerevisiæ*) is a well-known therapeutic agent and is a popular remedy for boils. In the Pharmacopœia of 1885 it was employed for poultices (*Cataplasma fermenti*).

For furunculosis drachm doses may be given two or three times a day. Landau and Albert have used vaginal injections of 10–22 c.c. in leucorrhœa. It has also been used in constipation, meteorism, and diabetes.

Since it contains large quantities of nuclein, a phosphorised proteid, yeast has been recommended in pthisis.[1]

In De Backer's method [2] pure cultures of yeast are stored under pressure in glass vessels, resembling soda-water siphons, from which the yeast can be injected hypodermically by means of a hollow needle attached to them. It is employed in the treatment of tuberculosis and cancer. The exact mode of preparation of the cultures does not seem to have been published.

For therapeutic use, it would be preferable to employ *pure* cultures of yeast, if obtainable.

---

[1] On the therapeutic use of yeast, see Ullman, *American Medicine*, October 11, 1902, p. 582; Merck's *Annual Rep.* for 1902, p. 64; *Rev. méd. de la Suisse romande*, 1901, No. 8.

[2] See *Brit. Med. Journ.*, 1897, ii. p. 802.

APPENDIX

1.—*Weights and Measures.*

1 cubic centimetre (1 c.c.) = 16 minims nearly.
10 cubic centimetres . = 2½ fluid drachms nearly.
1 litre . . . . = 35 fluid ounces nearly.
1 pint . . . . = $\frac{4}{7}$ litre or 568 c.c.
1 gram . . . . = 15½ grains nearly.
1 gram of dry serum . = 10 c.c. of fluid serum.

2.—*Physiological Salt Solution.*

This is a 0·7 per cent. solution of sodium chloride in distilled water (sometimes called 'normal saline solution').

3.—'*Normal*' *Solutions.*

By a 'normal' solution is meant the equivalent weight, in grams, of a substance dissolved in (*i.e.* made up to) a litre of distilled water; a 'deci-normal' solution $(\frac{N}{10})$ contains one-tenth of, a 'centi-normal' $(\frac{N}{100})$ one-hundredth of, a deka-normal' (10N) ten times, this amount. Thus, a normal solution of caustic soda contains 40 grams of pure $NaOH$ ($NaOH=40$), of sulphuric acid 49 grams of pure H_2SO_4 $\left(\frac{H_2SO_4}{2}=49\right)$ per litre.

4.—*Deterioration of Anti-sera.*

All anti-sera undergo a progressive diminution in strength, which is probably much more rapid in the case of anti-microbic sera than of anti-toxic sera. This deterioration is hastened by a high temperature and by the action of light; all sera should therefore be stored in a cool, dark place. Dried sera keep better than the fluid sera. Diphtheria and tetanus anti-toxins do not undergo any serious deterioration in a less period than six or nine months.

5.—*Firms supplying Anti-toxins, &c.*

Messrs. Allen & Hanburys, Vere St., Cavendish Sq., W.
Messrs. Burroughs, Wellcome, & Co., Snow Hill Buildings, Holborn Viaduct, E.C.
The Jenner Institute of Preventive Medicine, Chelsea Gardens, S.W.
Messrs. Meister, Lucius, & Brüning, 51 St. Mary Axe, E.C.
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Royal Veterinary College, Camden Town, N.W.

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